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Tumour bed positivity following breast-conserving surgery in breast cancer: risk factors and effect on patient outcome

By

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MBChB, FRCS (Glas)

Thesis submitted for the degree of Doctor of Medicine

RESTRICTED

UNIVERSITY OF GLASGOW

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ABSTRACT

This thesis is an exploration of certain aspects of breast-conserving surgery. It examines the risk factors as well as implications of local recurrence. All relevant published literature has been reviewed. The core of this thesis deals with a review of 752 patients who underwent breast-conserving surgery for invasive disease. Of these, 543 patients had tumour margin assessment performed by histopathological analysis of shavings from the residual cavity following initial wide local excision. The incidence of tumour bed positivity was 37%. A proportion of these patients underwent further surgery. At a mean follow-up of 52 months the local recurrence rate was 2%. Tumour bed positivity was found to be associated with symptomatic presentation; poorer tumour grade; lymphovascular invasion; extensive in-situ disease within the tumour and mammographically-detected casting calcification as well as absence of a nidus. Tumour bed positivity was found to predict for both poorer disease-free and distant disease-free survival. Furthermore, following introduction of tumour bed assessment the local recurrence rate fell from 16 to 2.5%. The implications of these findings are expanded upon in the following work.

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DECLARATION

I declare that I have composed this thesis and that the work contained herein was performed either by myself or in conjunction with others. Pathological analysis described in Chapters 1 and 2 was performed by Dr E Mallon, Department of Pathology, Western Infirmary, Glasgow. The immuno-histochemical staining, described in Chapter 4 was performed by myself with the assistance of Mrs R Ferrier, Department of Pathology, Western Infirmary, Glasgow. Dr E Mallon subsequently examined the prepared slides. The mammographic features described in Chapter 3 were interpreted by Dr L Wilkinson, Department of Radiology, Western Infirmary, Glasgow. Dr J Love at the Robertson Institute of Biostatistics, University of Glasgow, supervised statistical analysis on work performed. Presentations performed at scientific meetings pertaining to the work described in this thesis are listed below.

PRESENTATIONS BASED ON WORK CONTAINED

Malik HZ, Purushotham AD, Macmillan RD et al. *"Tumour bed positivity in women undergoing breast-conserving surgery for primary breast cancer"* presented at the Association of Surgeons of Great Britain & Ireland meeting in May 1998.

Malik HZ, Purushotham AD, Macmillan RD, Mallon E, Harnett A, George WD. "*Influence of "cavity shavings" on local recurrence following breast-conserving surgery*" presented at British Association of Surgical Oncology meeting in June 1998.

Malik HZ, Wilkinson L, Purushotham AD, George WD. "*Pre-operative mammographic features predictive of clinico-pathological risk factors for the development of local recurrence*" presented at the Association of Surgeons of Great Britain & Ireland meeting in May 1999.

Malik HZ, Purushotham AD, Macmillan RD, Mallon E, Harnett A, George WD. "*Influence of "cavity shavings" on local recurrence following breast-conserving surgery*" presented at the Nottingham international breast conference in September 1999.

PUBLICATIONS BASED ON WORK CONTAINED

Malik HZ, Purushotham AD, Mallon E and George WD. Influence of tumour bed assessment on local recurrence following breast-conserving surgery for breast cancer. *Eur J Surg Oncol* 1999; **25**: 265-268

Malik HZ, George WD, Mallon EA, Harnett AN, Macmillan RD, Purushotham AD. Margin assessment by cavity shaving after breast-conserving surgery: analysis and follow-up of 543 patients. *Eur J Surg Oncol* 1999; **25**: 464-469

Malik HZ, Wilkinson L, Purushotham AD, George WD. Pre-operative mammographic features predictive of clinico-pathological risk factors for the development of local recurrence. *Breast* 2000; **9**: 329-333

INTRODUCTION

EPIDEMIOLOGY OF BREAST CANCER

The incidence of breast cancer is higher in western countries than in the third world. When immigration occurs from a low to a high-risk country an increase in the incidence of breast cancer is observed (1). This risk is present within subsequent generations of the migrant population and reflects the incidence of the disease within the high-risk country (1). This suggests that there are social and environmental factors within a community, which may contribute to the incidence of breast cancer (1). Between 1983 and 1987 the world standardised incidence of breast cancer in Scotland was 62.60 per 100,000 of the population, compared to the incidence in England and Wales of 56.10 per 100,000 of the population (1). The incidence of disease in England and Wales is lower than most other developed countries, where the incidence of breast cancer ranges from 66.20 per 100,000 of the population in France to 89.20 per 100,000 in the U.S.A (1). However, the incidence of breast cancer is rising throughout the western world with 30,056 new cases being diagnosed in England and Wales in 1992 alone (1).

In 1992 breast cancer caused 13,663 deaths in England and Wales accounting for 4.8% of all female deaths (1). Apart from ischaemic heart disease breast

cancer was the largest single contributor to female mortality (1). These figures for breast cancer-related mortality translate to 39.47 deaths per 100,000 of the population within England and Wales alone (1). Deaths from breast cancer are higher within the U.K than most other western nations, with the exception of Denmark (1).

In 1991 the government published a white paper entitled *The Health of the Nation* in which strict targets and objectives were laid out in order to improve the nation's health care by the year 2000. One target set, was a 25% reduction in breast cancer-related mortality, especially within the screen-detected population. This would mean an U.K-wide reduction in breast cancer-related deaths from 93.6 per 100,000 in 1990 to 72.7 per 100,000 by the year 2000 (1).

In Scotland since 1959 there has existed through the Scottish National Cancer Registry a means whereby epidemiological data could be collected and analysed. The Scottish Cancer Trials Group has used such data to assess changes in the trends of breast cancer presentation and management over the past decade. From 1987 to 1993 there was an increase in the number of patients with breast cancer referred by General Practitioners to units specialising in the treatment of this disease (2). In 1987, specialist surgeons

saw 35% of new breast cancer cases compared with 57% in 1993 (2). A response by the government to reduce the mortality rate from breast cancer led to mass population screening being introduced for all women within the 50-64 year age group. Mass screening was initially introduced into Scotland in 1987 and to the rest of the U.K from 1991 onwards. In 1987 only 4% of breast cancers treated within the screening age range were screen-detected (2). However, in 1993 this figure had risen to 49% (2). Similarly in other European countries mass screening for breast cancer was introduced at the same period of time. In the Netherlands for example, in 1990 when screening started, 355 new cases of breast cancer were detected by this method (3). In 1993 however, this rose to 1450 new cases, a 17% increase on the non-screened figures (3). From 1997 onwards there is expected to be a steady 3.5% increase in tumour detection as a result of screening (3). Due to the increased detection of smaller operable tumours as a result of mass screening, there is expected to be a rise in the number of tumours treated by breast-conserving surgery. In the Netherlands between 1983-84, 33% of tumours were treated by breast-conserving surgery (3). This figure is expected to rise to 55% of all newly diagnosed breast tumours by the year 2000 (3). Despite these results screening has some limitations. Mushlin et al performed a meta-analysis of the results from several screening trials (4). This team found that the true-positive rate varied from only 83% to 95% (4). There was also a

significant false-positive rate of up to 6.5% that invariably would lead to unnecessary morbidity (4). Furthermore, screening is associated with significant radiation exposure (5). This has resulted in certain quarters questioning the validity of screening (6).

Within the Greater Glasgow Health Board district among the 35% of women within the screening age range, compliance of screening was 66% (2). Furthermore, compliance was not found to be affected by socio-economic status (2). As a direct impact of screening, there was an increased incidence of clinically stage I and axillary node negative tumours being detected (2). Due to a combination of screening and increased patient awareness of breast cancer, more patients were presenting earlier with smaller tumours, which were amenable to breast-conserving surgery (2). This factor along with an increased acceptance of breast-conserving surgery as an alternative to mastectomy by the surgical community, led to an increase in the use of breast-conserving surgery (2). In 1987, 40% of breast cancer cases were treated by breast-conserving surgery and this figure rose to 52% in 1993 (2). Along with the increased use of this surgical technique, there also occurred an increase in the number of patients being offered adjuvant therapy (2). In 1987, 70% of breast cancer patients were offered adjuvant therapy compared to 96% in

1993 (2). This rise was as a result of both an increased use of hormonal as well as cytotoxic chemotheraputic agents (2).

In summary breast cancer is a common form of cancer that forms a challenging clinical problem. Its incidence appears to be increasing. However, with the advent of screening resulting in earlier detection as well as an improvement in adjuvant therapeutic measures it is hoped that the mortality from this disease could be reduced. There has been data published that has shown a fall in the mortality from breast cancer (7). These results are most marked in the United States however (7). Furthermore, results from the Gothenburg screening trial have shown an improvement in the mortality rates of women as a result of screening thus validating the immense expense involved in undertaking such a mass screening programme (8).

HISTORY OF BREAST CANCER SURGERY

Although cases of breast cancer being treated surgically date back to Egyptian physicians in 3000 B.C, it was not until the mid-nineteenth century that with the discovery of antiseptics and anaesthesia, surgical management of breast cancer became a viable option. The discovery of the microscope, which led to the expanding science of cell biology, for the first time led pathologists to make a histological diagnosis of breast cancer.

In 1867, Charles Moore a surgeon in the Middlesex and St. Lukes hospital in London, published a paper in which he detailed what he believed were general principles on which breast cancer surgery should be based. In a study of 14 patients he noted that all local recurrences occurred at the site of the previous surgery (9). This led him to suggest that local recurrence was due to inadequate tumour excision (9). He concluded that the minimal amount of surgery that should be performed for cases of breast cancer should be a mastectomy. Charles Moore also advised an en-bloc dissection of the axilla in cases where nodal involvement was clinically suspected (9).

In the late nineteenth century, pathologists discovered the presence of tumour deposits within the pectorialis major muscle and fascia. This contributed

towards a call for more radical surgery in the management of breast cancer. In 1907 William Halsted published the results from 232 operations for breast cancer (10). Halsted routinely performed resection of the breast tissue along with a wide resection of the overlying skin and excision of the pectorialis major muscle. Following mastectomy the wound would be closed by skin grafting. He also performed resection of the axillary nodes in all patients. Halsted achieved a 3-year survival of 38.3% following his radical mastectomy procedure (10). This survival figure showed a halving of the mortality rates when compared with the results obtained by his contemporaries (10).

Towards the beginning of the twentieth century however, the efficacy of radical surgical procedures for the treatment of breast cancer were being questioned. In an overview of the previously published literature by Lane-Claypon in 1924, the results of 20,000 operations were analysed retrospectively (11). All patients who were lost to follow-up and those without a pathological diagnosis of breast cancer were excluded from this study. Although this study suffered from the hazards of a retrospective, non-randomised clinical trial, it did however show that the traditional Halsted mastectomy procedure was associated with a 29.2% three-year survival compared to a 43.2% survival for less radical forms of surgery (11).

The accepted theory about the spread of breast cancer in the late nineteenth century as proposed by Halsted, was that secondary tumour deposits occurred as a result of a non-breaking chain of tumour cells arising from the primary site and travelling along lymphatic channels to the site of distant disease (10). It was not until 1936 that this theory was questioned when pathologists showed the presence of anatomically normal lymphatic channels between the primary tumour and axillary nodal disease (12). In 1938 a modification of the traditional mastectomy procedure was proposed by Patey and Duson (13). They raised skin flaps in order to allow excision of the breast tissue followed by primary closure of the wound thus negating the need for skin grafting. This team also left the pectorialis major muscle intact. This operation was the forerunner of the simple mastectomy procedure commonly used to date.

With the advent of radiotherapy interest grew in less radical forms of surgery in order to reduce the extremely high rates of patient morbidity and mortality. An important breakthrough in the usage of radiotherapy came with the introduction of supravoltage radiation therapy equipment in the late 1950's. This equipment allowed for the first time a homogenous dosage of radiation to the breast with minimal radiation scatter to the surrounding organs. With the availability of radioisotopes, such as Irridium-192 the use of a boost dose to a selected part of the breast became possible. More recently with electron beam

irradiation it has become possible to perform a boost dosage on a more convenient outpatient basis.

The first clinical trial conducted showing comparable rates of survival between the traditional radical mastectomy and simple mastectomy (combined with post-operative radiotherapy) was started in Copenhagen in 1951 (14). This trial consisted of 666 patients prospectively randomised to either radical mastectomy or simple mastectomy followed by post-operative radiotherapy. In both cohorts only 76% of patients were treated according to the trial protocol due to either tumour in-operability or patient refusal of surgery. Despite this limitation however, no difference in patient survival was observed between these two cohorts (14).

Since the 1970's there have been five prospective randomised trials that have compared breast-conserving surgery to mastectomy (Table 1). One of the largest European trials was from the National Cancer Institute of Italy in Milan, this trial evaluated results on 701 women between 1971 and 1980 with primary tumours of 2cm or less (15). In this trial conservation surgery consisted of a quadrantectomy and a level 3 axillary dissection. Post-operative radiotherapy consisted of a 50Gy dose to the breast over 5 weeks followed by a 10Gy boost to the tumour bed. During the course of this trial a change to the

treatment protocol was introduced in 1975 when all patients with involved nodes were treated with adjuvant chemotherapy consisting of 12 cycles of CMF. Despite the potential inaccuracy introduced by this change in treatment protocol during the course of an on-going trial, the Milan trial found no survival advantage of mastectomy over breast-conserving surgery. Furthermore the breast-conserved cohort had a low local recurrence rate of only 0.3% (15).

The National Surgical Adjuvant Breast and Bowl Cancer Project (NSABP) began a three-armed trial (protocol B-06) in 1976 comparing mastectomy with segmental resection with or without radiotherapy (16). A total of 1843 patients with tumours up to 4cm in diameter were included in the study by its closure in 1984. All patients underwent axillary dissection. Those with positive nodes received adjuvant chemotherapy with Melphalan and 5-Flurouracil. Conservation surgery consisted of excision of the primary with enough surrounding tissue to ensure a tumour free inked margin. In 10% of patients however a positive margin was present and these patients went onto a completion mastectomy. Radiotherapy was delivered to the breast at a dose of 50Gy but without an additional boost to the tumour bed. The 8-year results from the NSABP trial showed that 39% of patients not receiving radiotherapy developed local recurrence compared to 10% in those who received post-

operative radiotherapy (16). However despite this difference in local recurrence rates no significant survival advantage of mastectomy over breast-conserving surgery was found (16).

The results from these trials lead to a change in surgical opinion favouring breast-conserving as a viable alternative to mastectomy. The implementation of mass screening as well as increased patient awareness of breast cancer has led to an increasing number of patients presenting with smaller tumours that are suitable for breast-conserving surgery (2). This, along with patients demanding a better cosmetic result following surgery has further increased the use of breast-conserving surgery as an alternative to mastectomy in the management of breast cancer. The increasing preference for breast-conserving surgery has been reflected in epidemiological data (2).

Breast-conserving surgery offers a better cosmetic result, reduced patient morbidity and a shorter in-patient stay. However, this type of surgery is dogged by one significant complication - that of recurrence within the ipsilateral breast. From Table 1 it can be seen that local recurrence rates following breast-conserving surgery vary from 0.3 to 15%. These diverse figures represent differences in both patient selection; the aggressiveness of

surgical excision for example quadrantectomy versus lumpectomy; as well as post-operative radiotherapy regimens within these varying trials.

Local recurrence not only undermines patient morale but may lead to mastectomy, thus defeating the initial purpose of breast-conserving surgery. More recently however, local recurrence is being seen by clinicians in a more sinister light as the harbinger of systemic recurrence. There are several recognised risk factors for the development of local recurrence that will now be explored in greater detail.

Table 1: Prospective randomised clinical trials comparing breast-conserving surgery and mastectomy

Trial	Number of patients	Tumour diameter (cm)	Radiotherapy regimen	Follow-up (years)	Overall survival	Local recurrence
MILAN I	701	<2	50 Gy 10 Gy boost to tumour bed	13	71% for Quadrantectomy 69% for Mastectomy	0.3% for Quadrantectomy
NSABP-B06*	1843	<4	50 Gy No boost to tumour bed	9	69% for Lumpectomy 68% for Mastectomy	12% for Lumpectomy
WHO	179	<2	45 Gy 15 Gy boost to tumour bed	10	77% for Lumpectomy 75% for Mastectomy	
NCI	247	< 5	48 Gy 15-20 Gy boost to tumour bed	10	77% for Lumpectomy 75% for Mastectomy	5 % for Lumpectomy 10% for Mastectomy**
EORTC	878	< 5	50 Gy 25 Gy boost to tumour bed	8	73% for Lumpectomy 71% for Mastectomy	15% for Lumpectomy 10% for Mastectomy**

- In all above trials there was no statistically significant difference in overall survival between the mastectomy and breast-conserving surgery cohorts

* Result at 10-year follow-up

** Loco-regional recurrence rates for patients undergoing a mastectomy

RISK FACTORS FOR LOCAL RECURRENCE

The identification of risk factors associated with the development of local recurrence following breast-conserving surgery is of potential use in refining patient selection criteria and treatment modalities. There are however significant methodological problems involved in assessing the risk factors associated with local recurrence in the breast.

There are a multitude of publications looking specifically at this issue. These vary from small retrospective studies to prospective trials of varying size. Most of these studies use differing statistical methods to analyse the data. Furthermore, the results obtained often vary in respect to the specifics of the type of breast-conserving procedure or adjuvant therapy used. For example, factors associated with local recurrence following quadrantectomy may well be different from those following a lumpectomy. Thus differences in patient selection, radiation dose, boost volume and the use of adjuvant systemic therapy will all have an influence on the factors found to be predictive of local recurrence within the breast.

CLINICAL FACTORS

Patient age and hormone replacement therapy

Young age has been found to correlate with increased risk of local recurrence in several studies (17-23). Of these however, only the study by Veronesi was a prospective randomised trial (17). In this study 579 women with pathological tumour diameters of less than 2.5cm were randomised to quadrantectomy with or without post-operative radiotherapy. Veronesi found that there was a significant fall in the local recurrence rate associated with older age (17). Women younger than 55 years had a local recurrence rate of 17.5% compared to only 3.8% in those aged over 55 (17). This study has however several limitations. Firstly, although Veronesi found that an extensive in-situ component within the tumour also predicted for local recurrence a multivariate analysis was not performed in order to identify whether age was an independent predictor of local recurrence. Furthermore, when assessing the impact of clinico-pathological factors on local recurrence Veronesi's team only assessed those 273 patients that underwent quadrantectomy alone rather than the total cohort of 579 patients. These factors further undermine the validity of the above results.

Kurtz performed a retrospective analysis of 586 patients treated by breast conserving surgery and found that on univariate analysis younger age predicted for local recurrence (19). However, this relationship was lost on performing a multivariate analysis (19). These results may well be biased by the fact that even when a positive margin was present no attempt at re-excision was performed. Furthermore, there is no formal mention of the numbers of patients with a positive margin and margin involvement was not included within the final multivariate analysis. Similarly Fourquet performed a retrospective analysis on 563 patients with "early" breast cancer and found that in both a univariate as well as a multivariate analysis younger age was a predictor of local recurrence, relative risk of 2.44 (20). Although this study provides the strongest evidence that younger age is a predictor of local recurrence, Fourquet's work is limited by the fact that it is retrospective in nature with no form of randomisation. Furthermore, the patients had been collected from 1960 to 1980, a time frame over which there would inevitably have been changes in the adjuvant therapy used. This is highlighted by the fact that only 53% of the patients received a radiation boost to the tumour bed (20).

The shortcomings of the above studies do suggest that the relationship between patient age and its risk for the development of local recurrence is

difficult to quantify. This is partly due to the fact that there are often only small numbers of patients who fall within the young age category, for example in the study by Sismondi only 35 patients were aged less that 35 thus making any subsequent statistical analysis subject to question (23). Also, most studies dealing with this issue have tended to use random and differing cut off between the "younger" and "older" patients further confusing subsequent analysis. Furthermore, younger age has been associated with the presence of poorer tumour grade; extensive intra-duct component within the tumour as well as the presence of lymphovascular invasion and inadequate tumour excision - all of which have been recognised as independent risk factors for the development of local recurrence (19-23).

For several decades oestrogens and progestagens have been prescribed to replace the cyclical production of ovarian hormones that normally ceases after the menopause. Several studies have shown that long-term use of hormone replacement therapy is associated with an increased risk for the development of breast cancer (24-25). The "collaborative group on hormonal factors in breast cancer" published a major work analysing the relationship between hormone replacement therapy and breast cancer (24). A meta-analysis was performed of 51 epidemiological studies and a total of 161,106 patients. This study found that the risk of developing breast cancer in women who were

greater than 5-years post-menopausal fell by 2.7% every year (24). Furthermore, those women on hormone replacement therapy, who had been on this medication for less than five years, the relative risk of developing breast cancer was 1.023 (24). In women taking hormone replacement therapy for greater than 5-years this relative risk rose to 1.35 (24). Although meta-analysis is a strong statistical tool and provides some useful information there are however some drawbacks to this type of study. Firstly, although a total of 21 countries were represented within this study the majority of the 51 papers examined were from the United States and Northern Europe. This inevitably leads to a bias. Also, the majority of women with breast cancer included within this analysis were diagnosed in the early 1980's. At that time the type of hormone replacement therapy used was predominantly oestrogen based with only 12% of women within this trial being on a combined oestrogen and progestrone prescription (24). Modern hormone replacement therapy prescriptions consist of combined preparations and thus their impact on the risk of developing breast cancer may well be totally different. Furthermore, there is little data available on the impact of long-term hormone replacement therapy on the risk of developing breast cancer.

Although there appears to be a relationship between hormone replacement therapy and breast cancer, Bonnier found that in those patients developing

breast cancer following hormone replacement therapy use, the tumours were usually smaller, less pleomorphic and tended to be node negative (26). Also, in this study no increased risk of either local or systemic recurrence among those tumours associated with hormone replacement therapy use was found (26). There is however one major weakness of this trial. The data presented was collected retrospectively and in a non-randomised manner between 1985 and 1995. Thus the differences in patient outcome seen between the hormone replacement therapy users and non-users may be down to chance.

Although the data published to date suggest that long term use of hormone replacement therapy is associated increased risk of developing breast cancer, it appears that these cancers are usually less aggressive. However, despite these findings there remains the need for further investigation into this subject.

Family history

Although between 5-10% of breast cancers are familial due to the relatively small numbers of such patients, there have been few studies dealing with familial breast cancer and its association with poorer outcome. Furthermore, most of these studies grade patient as having familial breast cancer based on hospital records and these may have an inherent inaccuracy.

Harrold et al published data suggesting that there was no relationship between familial breast cancer and an increased risk of local recurrence (27). In this study a retrospective analysis of 984 patients was performed. Of these patients 15% went onto develop local recurrence (27). However, of the total of 112 patents developing local recurrence, only 52 of these patients were chosen at random and included in the analysis of the risk of local recurrence. Furthermore, both the control and study group had significant variations in both tumour margin positivity as well as nodal status. This combined with the fact that any analysis of such small numbers has limited power makes this study inherently flawed.

In a study by Chabner et al a total of 201 patients younger than 36 years of age were analysed and those patients with and without familial breast cancer

were compared with each other (28). Chabner found that those patients with familial breast cancer had an increased of developing cancer in the opposite breast, relative risk of 5.7 (28). However, there was no association between familial cases and poorer local control or overall survival (28). Although these results are interesting, they are limited by the fact that of the 201 patients within the study only 29 had familial breast cancer. Furthermore, the criteria that were used to diagnose a patient with familial breast cancer within this study were the presence of a sister or mother with breast diagnosed before the age of 50 years. Thus this study underestimates the true incidence of familial breast cancer, as patients with more distant relatives as well as paternal relatives are not included within the analysis. Also, this study deals with only young patients with breast cancer and thus is unrepresentative of a vast majority of patients with breast cancer.

In summary there has been no good evidence that familial breast cancer is associated with increased risk of local recurrence. This may be explained by however by the small numbers of patients with familial breast cancer.

Mammographic features

In order to reduce the morbidity associated with a positive tumour margin necessitating re-excision, pre-operative identification of the risk factors for the development of local recurrence can be extremely useful in influencing the extent of primary surgery. The vast majority of patients with breast cancer undergo pre-operative mammograms. Macmillan et al performed a study in which pre-operative mammographic features were correlated with recognised clinico-pathological risk factors for the development of local recurrence (29). A total of 231 patients undergoing breast-conserving surgery had their pre-operative mammographic features assessed by a single radiologist blinded to the pathological data (29). In this study, an extensive intra-duct component within the tumour was significantly associated with the absence of mammographic nidus, presence of stellate nidus and casting micro-calcification (29). Furthermore, a dense mammographic pattern, casting calcification and absence of mammographic nidus all predicted for tumour bed positivity (29). All these features are recognised risk factors for the development of local recurrence (14,29-30). The limitation of the above study however is that the group of patients with each of the fore mentioned mammographic features is relatively small thus any statistical analysis of such data is weak. Also, the primary statistical tool used in this study was a

univariate analysis. This test is inherently weaker than a multivariate analysis, which if performed would identify those mammographic features that are independent predictors of local recurrence.

Liljegren et al performed a prospective randomised trial on 381 patients treated by breast-conserving surgery (30). In this study pre-operative mammographic features were recorded and were analysed separately as risk factors for the development of local recurrence. Liljegren found that on univariate analysis the presence of a stellate lesion with calcification was associated with an increased risk of developing local recurrence, relative risk of 3.8 (30). This factor correlates well with Macmillan's data. However, Liljegren also found that a non-stellate lesion and the absence of calcification was also associated with an increased risk of local recurrence, relative risk of 2.3 (30). This result appears to contradict Macmillan's data. However, when a multivariate analysis was performed the only mammographic feature that independently predicts for local recurrence was a stellate lesion associated with microcalcification, relative risk of 4.5 (30). Although the adequacy of tumour excision is important in local control, these pre-operative mammographic features may assist the surgeon in planning the extent of primary surgery.

TUMOUR FACTORS

Tumour diameter

Many studies have analysed the relationship between tumour diameter and the risk of local recurrence. In the EROTC trial when tumours greater or less than 2 cm in diameter were compared, increasing tumour diameter was not found to be a predictor of local recurrence (31). This trial consisted of 902 patients with stage I and II cancers randomised to either breast-conserving surgery or mastectomy. This study has several disadvantages however. Firstly any patients with a positive tumour margin were excluded from the trial and this will inevitably bias the subsequent results. Furthermore, analysis of tumour diameter as a predictor of local recurrence was based on a clinical measurement, which is inherently inaccurate. Similarly, in the Marseilles study no association was found between tumour size and local recurrence (19). This study was performed in a retrospective manner analysing the outcome of 496 patients treated by breast-conserving surgery (19). Although a positive margin was found in 10% of cases and in a further 37% of cases a confident assessment of the margin status could not be performed, no attempt at re-excision was made (19). This finding suggests that in a vast proportion of the patients within this trial the primary surgery was inadequate and this fact may well have biased the subsequent results.

There have been several studies that have found a positive correlation between increasing tumour size and local recurrence (Table 2). One major study published from the United States was the NSABP-B06 trial (16). A total of 1843 patients with tumours up to 4cm in diameter were included in the study. Conservation surgery consisted of excision of the primary with enough surrounding tissue to ensure a tumour free inked margin. In 10% of patients a positive margin was present and these patients went onto a completion mastectomy. On performing a univariate analysis pathological tumour size was found to predict for local recurrence (16). This relationship remained significant on a subsequent multivariate analysis, relative risk of 1.57 (16). There is however one flaw within the trial design that may have biased these results however. Namely the 10% of patients who underwent a completion mastectomy for a positive margin were included in the breast-conserved cohort for the final analysis. This fact makes interpretation of the results from this trial fraught with difficulty.

Veronesi et al performed a retrospective study between 1970 and 1987 on 2233 patients undergoing quadrantectomy and axillary clearance followed by radiotherapy to the breast (32). Pathological tumour size was subsequently stratified into four groups. This study showed that on a multivariate analysis the largest tumours (>2cm) predicted for local recurrence when compared to

tumours less than 0.5cm, relative risk being 3.159 (32). It should be noted however that the breast-conserving procedure adopted by Veronesi consists of a quadrantectomy and is more extensive a resection than used by most teams. Thus the results from this trial cannot be easily extrapolated to patients who have been treated by more limited primary surgery.

Holland et al looked at the issue of tumour multifocality by serially sectioning 282 mastectomy specimens of patients suitable for breast-conserving surgery (33). This group found that at a 2cm margin from the primary tumour 43% of cases had residual disease (33). This figure correlates well with the local recurrence rates of the "lumpectomy alone" arm of the NSABP-B06 trial (16). Furthermore, there was no association between tumours less than or greater than 2cm and an increased incidence of residual microscopic disease around the tumour (33). This suggests that the association between tumour diameter and local recurrence may simply reflect the fact that larger tumours make a complete surgical excision more difficult. Thus any correlation between tumour diameter and local recurrence may merely be due to the adequacy of local excision protocols practised within each of the above studies.

Table 2: Tumour size versus local recurrence

Study	*P*-value	Relative Risk
NSABP-B06	0.001	1.57
Veronesi et al	0.0183	3.159
Deore et al	<0.05	Not available
OCOG	0.01	1.64
Bonnier et al	0.005	1.75

- *p*-values represent results of analyses comparing increasing tumour size and risk of local recurrence

Extensive intra-duct component within tumour (EIC)

Several clinical trials have shown that an extensive intra-duct component within the tumour (EIC) to be an independent risk factor for the development of local recurrence (17,32, 34-37) (Table 3). However, one factor that makes a comparison of these various studies difficult is the fact that often different researchers used differing definitions for EIC. One widely accepted definition of EIC is greater than 25% of the primary tumour containing in-situ disease (37). In the Milan III trial following quadrantectomy, the local recurrence rate fell from 16.7% for patients with EIC to 7.4% in those without EIC (17). This trial however has two serious design flaws. Firstly, all patients with a positive margin were excluded from the study. Secondly, the analysis of the risk factors for the development of local recurrence was performed in only the 273/567 patients within the trial who did not receive adjuvant post-operative radiotherapy. Similarly, Veronesi's review of 2233 women treated by quadrantectomy between 1970 and 1987 showed that the presence of EIC predicted for local recurrence (32). Interestingly, although a total of 119 patients developed local recurrence, the final analysis was only performed on 115 patients, as 4 patients who developed local and systemic recurrence spontaneously were re-grouped within the systemic recurrence cohort (32). Also of note is the fact that of the 119 patients with a local recurrence

histological information was present on only 100 patients and thus only a proportion of patients with local recurrence could be assessed for the presence of EIC (32). Furthermore, this team did not provide a definition of EIC and the pathology was not reviewed by a single pathologist thus the criteria that this team used to define patients as having EIC are open to question. It should also be noted that in both the above trials patients underwent a quadrantectomy, which involves a more extensive resection than commonly used. Thus it is may not be possible to extrapolate this data to patients undergoing more limited resections.

Sinn et al reviewed a total of 957 patients treated by breast-conserving surgery between 1985 and 1992 (34). All the histological slides were reviewed and re-graded. Interestingly Sinn's definition of EIC was related to the ratio of the in-situ component to the invasive component. Tumours were graded as having either EIC if the in-situ component was two times the size of the invasive component or as having a predominant intra-duct component (PIC) if the in-situ disease was four times the size of the invasive disease (34). In this study the local recurrence rate was 6% (34). On a univariate analysis, the presence of EIC/PIC was associated with local recurrence (34). This relationship remained significant when a multivariate analysis was performed, relative risk of 1.90 (34). A similar study by Borger et al, where the pathology

of 969 patients who underwent breast-conserving surgery was reviewed and outcome analysed, revealed that EIC was a predictor of local recurrence (36). This relationship did not however remain significant on a subsequent multivariate analysis. Similar results were found in the Marseilles trial where EIC was found to predict for local recurrence (37). Interestingly the presence of in-situ disease at the edge of the tumour was also associated with local recurrence (37). However, this relationship may well be biased by the fact that in the vast majority of cases in-situ disease within the periphery of the tumour, EIC is also present.

The only major trial where no association between EIC and local recurrence was found was in the NSABP-B06 trial (38). One possible explanation for this may be that patients who had a mastectomy performed for positive resection margins were included in the analysis as part of the cohort treated by breast-conserving surgery. Thus the data from this trial may well be skewed.

In summary there have been a number of studies that have shown EIC to be a risk factor for local recurrence. Furthermore Sinn et al showed that the relationship between EIC and local recurrence is also determined by the relative proportional size of the in-situ to the invasive component. This relationship may be due to the fact that a large intra-duct component

surrounding the tumour may make an adequate tumour excision more difficult, thus increasing the risk of local recurrence. There is also data becoming available that shows a relationship between the grade of EIC and the risk of local recurrence. In a major study by Voogd et al, a total of 7000 breast-conserved patients from 11 centres throughout the Netherlands were analysed (39). A local recurrence rate of 5% was found (39). All patients had the pathology independently reviewed. EIC was defined as being present when 10 or more ducts within the primary tumour were involved with in-situ disease (39). Patients with EIC were subsequently subdivided into those with "high grade" comedo or "low grade" cribriform in-situ disease. The presence of "low grade" EIC was associated with local recurrence, relative risk of 1.9 (39). However, an even stronger association was found between "high grade" EIC and local recurrence, relative risk of 3 (39).

Table 3: Presence of EIC versus local recurrence

Study	*P*-value	Relative Risk
Milan III	0.04	Not available
Veronesi et al	0.0246	1.877
Sinn et al	<0.001	1.9
Leborgne et al	0.0016	Not available
Borger et al	0.025	Not available
Marseille trial	<0.001	Not available
BORST	<0.01	3
Lindey et al	0.003	Not available

- *p*-values represent results of analyses comparing EIC and risk of local recurrence

Tumour grade and type

The presence of poorer tumour grade has been shown in several studies to be an independent risk factor for the development of local recurrence (19,21,30-31,34,38-40)(Table 4). Sinn et al reviewed the pathological data of 957 patients treated by breast-conserving surgery and found that on performing a multivariate analysis, poorer tumour grade predicted for local recurrence, relative risk being 1.76 (34). The important feature of this study is that in all patients, the pathology was reviewed by a single independent pathologist. This avoids the risk of inter-observer variation. In the NSABP-B06 trial, on univariate analysis poorer tumour grade was found to predict for local recurrence, relative risk being 1.5 (38). This relationship did not remain significant on multivariate analysis however (38).

In Kurtz et al's review of 586 breast-conserved patients the presence of poorer tumour grade was found on multivariate analysis to be a risk factor for the development of local recurrence (19). Interestingly, this relationship was only significant among patients aged 50 years or older (19). Similarly in the review by Bonnier et al of 1266 patients treated by breast-conserving surgery between 1976 and 1992, the presence of poorer tumour grade was a predictor of local recurrence, relative risk of 2.53 (21). Again, this relationship was only

significant in-patients older than 35 years of age (21). This age related distribution may be explained by the fact that there are relatively few young patients with these studies, for example in Bonnier's cohort of 1266 patients only 93 were younger than 35 years of age (21). Thus any underlying statistical relationship may not become apparent unless larger numbers of patients are recruited within the younger age ranges. In the analysis by Voogd et al on 7000 patients, there was a total of 360 local recurrences (39). When the risk factors for the development of local recurrence were analysed comparing younger pre-menopausal to older patients, the presence of a poorer tumour grade was a significant risk factor in both cohorts (relative risk of 1.5 for the pre-menopausal compared to 1.7 for the post-menopausal patients) (39). The overall relative risk for the development of local recurrence for higher-grade tumours was 2 (39). One limitation of this study however, is the fact that tumours were graded according to the mitotic index rather than the Bloom and Richardson system that is common place today (39).

Approximately 80-90% of all breast cancers are ductal in nature. There have been several publications to date that have analysed the relationship between tumours of special type and the risk of local recurrence following breast-conserving surgery (34,38,40-41). In a pathological review performed by Kurtz et-al on 861 stage I and II tumours treated by breast-conserving surgery,

the local recurrence rate was 13.5% at 5 years for lobular carcinoma compared with 8.8% for ductal carcinomas (41). However, this difference was not shown to be statistically significant (41). This may however be explained by the fact that within this study there were only 67 patients with lobular carcinoma (41). Similarly in the study by Lindey et al there was no association between tumours of special histological type and local recurrence (40). Again the limiting factor in this study is the fact that the numbers of such tumours are limited (40). In Sinn's review however, there was a relatively large series of lobular carcinomas amounting to 14.2% of the total number of patients included within the study (34). Sinn et al found that the 5-year local control in invasive ductal carcinomas was 94.8% compared to 88.2% in the patients with lobular carcinoma (34). It was noted however that this difference in outcome took 3 years of follow-up to become apparent (34). This may be explained by the fact that the early local recurrences, within the first 3 years, are as a result of inadequate tumour excision, which can occur in both these cohorts. However, after 3 years the poorer local control within the patients with lobular carcinoma may reflect the multicentric nature of this tumour. Similar results were found in the NSABP-B06 trial where lobular carcinomas were found to be a significant risk factor for the development of local recurrence, relative risk of 2.13 (38).

In summary poorer tumour grade has been found to be a risk factor for the development of local recurrence. This relationship appears to be most marked in older patients. However, this may merely representative of the difficulty of obtaining sufficient numbers of young patients with breast cancer in-order to perform a meaningful analysis.

When the 10-15% of patients with breast tumours of special type are analysed separately, it appears from the studies published to date that breast-conserving surgery is inappropriate for lobular carcinomas. This may be due to the multifocal nature of lobular carcinomas that mitigates against a breast-conserving procedure providing adequate tumour excision.

Table 4: Poorer tumour grade versus local recurrence

Study	*P*-value	Relative Risk
Kurtz et al	0.001	Not available
Bonnier et al	0.01	2.53
EORTC	<0.001	2.28
Sinn et al	<0.0001	1.76
NSABP-B06	0.0005	1.5
Voogd et al	<0.05	2
Lindey et al	0.0003	3.61
Liljegren et al	<0.05	2

Lymphovascular invasion

The presence of invasion by breast cancer cells of the vascular bundle can be a method of tumour dissemination independent of the usual method of spread through the lymphatic channels and eventually to the axillary/internal mammary nodes. Lymphovascular invasion is commonly present in approximately 22% of breast cancers (34,43). Several studies have however shown that the presence of lymphovascular invasion is also a predictor of local recurrence (28,30,32,34,36,38,42-43).

Chabner et al reviewed the data on 201 patients treated by breast-conserving surgery (28). All the histological slides were reviewed by two independent pathologists. Chabner found that lymphovascular invasion was a predictor of local recurrence, relative risk being 2.5 (28). This study has two main limitations however, firstly the numbers of patients included are relatively small with only 70 patients having lymphovascular invasion. Secondly, the patients included within this study were all aged 36 years or younger, thus it may not be possible to extrapolate these results to the vast majority of patients with breast cancer. Similarly Liljegren et al found that lympholvascular invasion predicted for local recurrence, relative risk 1.7 (30). Again, this study is hampered by the relatively small numbers of patients that were recruited

into this trial. Furthermore, the post-operative radiotherapy regime did not include a boost to the tumour bed and this fact may also have biased the above results.

Veronesi's review of the Milan data on 2233 patients treated by quadrantectomy found that local recurrence rate at 10 years in those patients exhibiting lymphovascular invasion was 26% compared to 6% in the remaining patients (32). Although this difference appears striking, unfortunately the presence of lymphovascular invasion was not included in any subsequent analysis of the risk factors for local recurrence. Thus Veronesi's results fail to shed any further light on the relationship between lymphovascular invasion and local recurrence.

The strongest data supporting the link between lymphovascular invasion and local recurrence has come from four separate studies (34,36,42-43). Sinn et al reviewed data on 957 patients treated by breast-conserving surgery of whom all the pathological data was reviewed independently (34). Among this cohort of patients, 203 (21%) had vascular invasion present (34). The presence of vascular invasion was found on a univariate analysis to be a strong predictor of local recurrence (34). When a multivariate analysis was performed vascular invasion remained a risk for the development of local recurrence, relative risk

of 1.34 (34). Borger et al reviewed 969 patients treated by breast-conserving surgery (36). On performing a multivariate analysis lymphovascular invasion predicted for local recurrence (36). Similar results were found by Cowen et al (42). In the study by Pinder et al a large cohort of 1704 women treated by breast-conserving surgery were followed up (43). The incidence of lymphovascular invasion was 22.8% (43). This group found that on a multivariate analysis lymphovascular invasion was a predictor of local recurrence, relative risk of 3.18 (43).

The above results have however been refuted by other studies (39-40). In the BORST trial, 7000 women treated by breast-conserving surgery throughout 11 centres within the Netherlands were followed between 1980 and 1994 (39). There were a total of 360 local recurrence (39). The presence of lymphovascular invasion was not found to be an independent predictor of local recurrence (39). Although the numbers of patients included within this trial are large, pathological data was only reviewed on a fraction of the total numbers. Among the local recurrences, in only 326 (91%) patients were pathological slides available (39). Furthermore, of the 6640 patients who did not develop local recurrence pathological data was reviewed on only 716 (10.7%) of these patients (39). Thus the final statistical analysis was

performed on a subgroup of the total numbers of patients thus biasing the final results.

In summary, there have been several studies that have shown a relationship between lymphovascular invasion and local recurrence. Although the presence of vascular invasion could be easily seen to predict for distal tumour spread, its association with local recurrence is more difficult to explain. The presence of tumour foci within blood vessels may be an indicator of wider local spread within the breast, thus reducing the effectiveness of wide local excision. This hypothesis seems to be backed by the data from the NSABP-B06 trial where the presence of lymphovascular invasion was a predictor of local recurrence among the patients treated by lumpectomy alone (38). This effect was negated however by the use of post-operative radiotherapy (38).

Inflammatory response to tumour

The presence of an inflammatory infiltrate surrounding a breast cancer has occasionally been described by pathologists. There have however been relatively few studies analysing the relationship between this immunological reaction to the tumour and patient outcome, especially local recurrence. Lindey et al reviewed 293 patients with breast cancer treated by breast-conserving surgery (40). This team found that in their series there was a 12% incidence of a moderate or extensive chronic inflammatory response to the presence of tumour (40). The presence of this infiltrate was significantly associated with local recurrence, relative risk of 2.86 (40). In the Marseille trial the presence of a marked lymphocytic infiltration was also found on multivariate analysis to be a predictor of local recurrence (19). This relationship appeared to be independent of patient age (19). Similar results have been found by Kurtz et al (44).

Unfortunately, due to the relatively small numbers of patients demonstrating this reaction, the data available to date consists of few scanty reports involving a limited numbers of cases. Thus it is as yet not possible to determine confidently any possible relationship between this factor and local recurrence.

Biological markers

The presence of oestrogen or progesterone receptor positivity in breast cancer is a valuable prognostic indicator. With the presence of oestrogen receptor positivity, the response to endocrine therapy can be expected in approximately 60% of cases (45). This figure rises to 75% when both the oestrogen and progesterone rectors are positive (45). Despite the presence of routine testing for oestrogen receptor status over the past decade, there are very few studies dealing with the issue of whether oestrogen receptor status affects local recurrence. Furthermore, any such studies performed have shown conflicting results.

Yughan et al reviewed pathological data on 256 patients undergoing breast-conserving surgery and found that oestrogen receptor negativity was a predictor of local recurrence (46). This relationship remained significant after a multivariate analysis (46). This study does however have several limitations. Firstly the numbers of patients included within the study is relatively small. Furthermore, the local recurrence rate within this study was 27% which is significantly greater than most of the published data. This finding reflects the fact that patients included within this study were recruited between 1984 and 1991 and over this period of time there would have been major changes in

treatment protocols. This is confirmed by the fact that only 30% of patients received post-operative radiotherapy and this fact almost certainly would have biased Yaghan's results. Vollenweider-Zerargini et al reviewed histological data on 547 patients in order to correlate receptor status with outcome (45). This team showed that oestrogen receptor positivity predicted for an improved disease-free survival (45). The presence of both oestrogen as well as progesterone positivity predicted for improved survival, however their presence did not predict for an improvement in disease-free survival (45). However, several studies have refuted these results (43,47-48).

Over the last decade there has been extensive research into the molecular biological characteristics of tumour that predict for relapse. Several factors have been identified as predictors of tumour recurrence. One such factor is Ki-67 (49). Aysegul et-al used anti Ki-67 antibodies and found that over-expression of Ki-67 predicted for poorer tumour grade and younger age, both of which are recognised risk factors for local recurrence (50). Furthermore, several studies have found over-expression of Ki-67 to be a predictor of local recurrence (49-50). However, these studies are hampered by the fact that they include relatively small numbers of patients.

Hartmann et al found in a study of 354 patients, that over-expression of the c-erb B2 oncogene resulted in a fall of the disease-free survival from 7.1 years to 2.9 years (51). Over-expression of c-erb B2 was also associated with axillary nodal disease, higher nuclear grade and oestrogen receptor negativity (51). Similar results have been found by Seshadri et al (52). This team also found an association between c-erb B2 over-expression and a shorter time to recurrence of the breast cancer (52). When a multivariate analysis was performed however, over-expression of c-erb B2 oncogene was associated with a worse disease-free survival only in the presence of nodal positivity (52). Gusterson et al showed on a study of 1506 patients that the absence of c-erb B2 expression was associated with a protective effect against tumour recurrence (relative risk of 0.57 in node positive and 0.82 in node negative patients) (53). It was also noted that over-expression of c-erb B2 was associated with a poorer response to the cyclophosphamide, methotrexate and 5-flurouracil chemotheraputic regime (53). However, other researchers have found that among patients over expressing c-erb B2 the use of high dosage regimens including doxyrubacin can overcome this resistance (6). In summary the ability to measure specific gene products in breast cancer offers the potential to predict the biological behaviour of an individuals disease, thus allowing the specific targeting of adjuvant therapy.

TREATMENT FACTORS

Inadequate surgical excision

One major risk factor for the development of local recurrence is believed to be the presence of residual disease left behind at the time of primary surgery. However despite the numbers of studies dealing with this subject, the analysis of their results is fraught with difficulty. This is partly due to the fact that in different studies varying techniques of surgical excision are employed. Furthermore, most of these studies have been performed over varying lengths of time. This invariably means that there have been differing adjuvant therapies available, which have an impact on the eventual results. Also, despite set protocols regarding surgical excision there is often no consideration of the impact of differences in inter-surgeon variation when the final analysis of these trials is performed.

Fisher et al showed that 86% of local recurrences occurred within the same quadrant as the primary tumour (54). Furthermore, these recurrences were of the same grade and histological type as the primary (54). Haffty et-al reviewed the results of 990 patients treated by breast-conserving surgery (55). This team showed that 57% of local recurrence were of the same pathological grade and type as well as within the same location within the breast as the

primary tumour (55). However, when DNA flow cytometry was performed there was a poor correlation between the local recurrence and the primary tumour (55). This may be explained by the fact that there were only 33 patients analysed by this technique. These small numbers of patients reduce the validity of Haffty's results. In the Milan trial, quadrantectomy was compared to a wide local excision of the tumour including a 1cm margin of grossly normal tissue (56). This study found that of those patients undergoing a wide local excision, 16% had positive resection margins compared to 3% of those undergoing quadantectomy (56). Furthermore, quadrantectomy was associated with lower local recurrence rates compared with more limited resection (reduction from 7% to 2.2%) (56). This clearly demonstrates the benefit achieved from more complete tumour resection.

The incidence of incomplete surgical excision of the breast primary is difficult to determine. The surgeon is often faced with a tumour that has a large and eccentric surface area. When this is combined with the fact that the tumour is often associated with irregular EIC extension, the possibility of inadequate tumour clearance exists. In the NSABP-06 trial the local recurrence rate was 43% among patients undergoing wide-local excision but not receiving post-operative radiotherapy (38). Holland et al showed that residual disease is found in 43% of cases (within a 2cm rim of grossly normal tissue surrounding

the tumour) (33). This figure approximates the local recurrence rate observed in the NSABP-B06 trial and indirectly contributes to the hypothesis that inadequate tumour excision predisposes to local recurrence. The problem of adequacy of tumour excision following breast-conserving surgery has led to various techniques that attempt to determine the completeness of tumour excision.

Several techniques have been described ranging from assessment of the lumpectomy specimen margin either by inking or touch preparation cytology (57-58) to the use of cavity shaving biopsies. Assessment of tumour margin clearance by the India ink technique involves taking multiple sections from the lumpectomy specimen and observing whether the tumour crosses or lies in close proximity to the inked margin. Unfortunately, there is no consensus regarding the definition of a positive tumour margin using this technique. Typically margin involvement is seen in 10% of cases (38). This value as demonstrated by Holland's data is an underestimate of the extent of residual disease (33). Furthermore, there is often significant inter-observer variation seen in this technique as demonstrated by Fisher's result where in only 31% of cases was there a consensus regarding positive margins between the hospital pathologist and the trials specialist pathologist (54). Furthermore, in a study by Schmidt-Ullrich et al between only 46-60% of patients undergoing a re-

excision following a positive inked margin (defined as tumour less than 2mm from the inked margin) had disease within the re-excised specimen (59). Also, 6% of patients had a re-excision because the tumour margin could not be assessed (59).

The method of touch preparation cytology has been described by Cox et al (57). Cox found that 18% of patients had a positive margin as assessed by this technique (57). In another study Cox showed that this technique was associated with 100% sensitivity and a 96.6% specificity (57). The advantage of this technique is that it takes approximately 15 minutes to obtain a result thus allowing a re-excision to be performed during the first operation, thus avoiding the need for re-hospitalisation. This technique does however have several limitations. Errors can occur due to specimen irregularity, diathermy artefact as well as diagnostic misinterpretation. More importantly false negative results have also been seen in tumours of special type for example lobular carcinoma (57).

The technique of cavity shavings involves excision of further tissue from the wall of the residual cavity following initial wide local excision. This tissue is subsequently analysed for the presence of residual disease (60-61). The method of taking a shaving of the residual cavity is a simple technique that is

less laborious and allows assessment of the degree of residual invasive or in-situ disease. Macmillan et-al reviewed the results of 264 patients undergoing breast-conserving surgery and showed that residual invasive disease within the tumour bed was found in 39% of patients (61). This figure correlates well with Holland's results (33). This group practised a policy of selective further surgery. In all 36 (13.6%) of patients underwent further surgery, 50% of whom had residual disease present within the re-excised tissue (61). This team found a local recurrence rate of 2% at mean follow-up of 4.4 years (61). This local recurrence rate is favourable when compared with the published literature (17,38,62-75). Guidi et al directly compared the techniques of inking and cavity shavings (60). In this study a total of 22 lumpectomy specimens were inked then a shaving from the surface of the inked margin was taken and analysed (60). From these 22 lumpectomies a total of 199 blocks were obtained (60). A total of 69 (34.7%) blocks showed a positive shaved margin (60). Of these only in 42 (61%) of cases was an inked margin present (60). This apparent discrepancy can be accounted for by the intrinsic differences in the two techniques. Inking involves selective evaluation of the surface of the specimen and in a large specimen this can be associated with a sampling error. The technique of cavity shaving however allows examination of a relatively large proportion of tissue surrounding the lumpectomy with relatively few histological sections. Furthermore, cavity shaving may also detect the

presence of multifocal disease as well as inadequate excision thus explaining the greater incidence of detecting disease with the use of this technique.

In summary, several studies have shown that local recurrence commonly arises as a result of inadequate tumour excision. In order to assess the adequacy of tumour excision several techniques have been developed. All the techniques developed to date including cavity shavings and inking of margins have strengths as well as weaknesses. Commonly a positive inked margin is found in 10% of cases compared to tumour bed positivity being present in 39% of cases. Among the institutions that routinely assess the tumour margin the presence of a positive margin invariably leads to further surgery. Thus not surprisingly there has as yet not been any direct statistical evidence linking a positive margin with local recurrence. However, of these institutions that have published their results, assessment of the tumour margin has resulted in a relatively low local recurrence rate (57,59,61).

Post-operative radiotherapy

The use of ionising radiation in medical practice has been established since the late nineteenth century. It was not till the late 1950's that the physiology of radiotherapy was better understood. Ionising radiation has both a direct effect on important molecular structures within tissues as well as an indirect effect where ionised radicals produced as a result of radiation cause damage to important biological structures.

With the advent of radiotherapy interest grew in less radical forms of surgery in order to reduce the extremely high rates of patient morbidity associated with more radical forms of surgery. An important breakthrough in the usage of radiotherapy came with the introduction of supravoltage radiation therapy equipment in the late 1950's. This equipment allowed for the first time a homogenous dosage of radiation to the breast with minimal radiation scatter to the surrounding organs. With the availability of radioisotopes, such as Irridium-192 the use of a boost dose to a selected part of the breast became possible. More recently electron beam irradiation it has become possible to perform a boost dosage on a more convenient outpatient basis.

The use of post-operative radiotherapy has been fundamental in the wider acceptance of breast-conserving surgery as a safe alternative to mastectomy. Several studies have shown the benefit of post-operative radiotherapy in reducing local recurrence (17,38,62-80). Some of this data is represented in Table 5 (17,38,62-64). As can be seen all these studies differ in design; patient population; follow-up; clinico-pathological patient factors as well as the extent of both surgical excision as well as radiotherapy regimens. However despite these factors there is overwhelming evidence that supports the use of post-operative radiotherapy in reducing local recurrence following breast-conserving surgery.

One of the major European trails that analysed the efficacy of radiotherapy was published in the 1993 by Veronesi (17). In this trial 567 with cancers smaller that 2.5cm were randomised to quadrantectomy with or without post-operative radiotherapy (17). The radiotherapy regime consisted of 50Gy to the whole breast over 5 weeks followed by a 10Gy boost to the tumour bed (17). Veronesi found that those patients treated with radiotherapy had a low local recurrence rate of 0.3% compared to 17.5% for those who did not receive radiotherapy (17). In this study the primary surgery used was a quadrantectomy. This is more extensive than that used in most institutions. Thus Veronesi's results may not reflect the data as a whole.

There have been two major trials published from North America that have been influential in showing the benefits of post-operative radiotherapy. Fisher et al published the 10-year results of the NSABP-B06 trial in 1991(38). This trial consisted of 1857 patients with breast cancers, measuring 4cm or less, that were randomised to either mastectomy or lumpectomy alone or lumpectomy and post-operative radiotherapy (38). The local recurrence rate for those patients not receiving radiotherapy was 43% compared to 12% for those receiving post-operative radiotherapy (38). These results may have been biased by the fact that within this trial the post-operative radiotherapy regime consisted of 50Gy to the whole breast, however no boost dose was given to the breast (38). Several studies have shown that not providing a boost to the tumour bed is associated with poorer local control (81-82). Interestingly between year 1 to 3 of the follow-up the annual hazard rate of developing local recurrence in the lumpectomy alone cohort was 8.5% compared to 4.6% for years 4 to 10 of the follow-up period (38). This difference almost certainly represents inadequacy of tumour excision. There was no such variation seen in the radiotherapy cohort suggesting that radiotherapy is protective against residual disease.

In 1994 Whelan et al published the results from the other major North American trial (62). A total of 837 women with node negative breast cancer

were randomised into this trial (62). The local recurrence rate in the no treatment arm was significantly higher compared to those patients that received post-operative radiotherapy (30% versus 8%) (62).

Although there is a wealth of data proving the efficacy of post-operative radiotherapy in improving local control, there has been evidence however that the type of radiotherapy protocol may also have an influence outcome. Randomised trials have shown that not giving a booster dose to the tumour bed is associated with poorer local control (81-82). In a study by Romestaing et al, 1024 women with breast cancers were randomised after initial wide local excision and radiotherapy to the breast (50Gy) to receive an additional 10Gy boost to the tumour bed (81). At a mean follow-up of 3.3 years the local recurrence rate within the boost cohort was 3.6% compared to 4.5% in the no boost cohort (81). Although this study contains a large number of patients the follow-up is relatively short and a longer follow-up would be required to ensure that this difference in the local recurrence rates is maintained over a longer period.

Deore et-al studied the effect of dose rate in post-operative radiotherapy on outcome in 270 patients (83). This team found that local recurrence was associated with implant dose rates less than 30 cGy/hr (83). It was also noted

that increasing the implant dose rate greater than 100 cGy/hr was associated with a poorer cosmetic result (83). Other studies have found similar results (84-85).

Meek et al studied the impact of a delay in post-operative radiotherapy and its impact on local control (86). In this study a total of 300 patients were recruited between 1984 and 1989 (86). Of these, 247 patients underwent post-operative radiotherapy with a mean delay of 8 weeks compared to 24 weeks in the "delayed" cohort. This delay was due to patients receiving adjuvant chemotherapy. Thus not surprisingly those patients who had delayed radiotherapy were younger and node positive compared to those patientsin whom radiotherapy was not delayed. Despite the differences in the clinico-pathological features between these two cohorts there was no significant difference in the local recurrence rate. This study however is limited by two factors, firstly the numbers included in this study are relatively small and secondly there has been no form of randomisation. Thus the results from this study are difficult to interpret. Leonard et al also analysed the relationship between a delay in post-operative radiotherapy and local control (87). In this study 262 patients were reviewed of whom in 105 patients radiotherapy was delayed due to chemotherapy (87). Leonard found that there was no significant difference in the local recurrence rate at 5-years between the two

cohorts (87). Furthermore, there was no time interval between surgery and radiotherapy that affected the local recurrence rate (87). Unfortunately this study suffers from the same limitations as Meeks's work. In order to analyse the effect of radiotherapy regimes on local recurrence there is the need for a meta-analysis.

In summary post-operative radiotherapy has been shown to have had an effect in reducing the local recurrence rates following breast-conserving surgery. This effect has been confirmed in meta-analysis of the published data (88). This publication by the "early breast cancer trialists' collaborative group" reviewed the results of 36 trial containing a total of 17,273 patients (88). Radiotherapy was associated with a lower local recurrence rate (88). Interestingly radiotherapy had been shown to results in a fall in the breast-cancer-related deaths (88). This result has been reproduced by Haybittle et al (89). The fall in breast cancer-related deaths following post-operative radiotherapy may well represent the impact of a decrease in the local recurrence rate among those receiving radiotherapy.

Table 5: Effect of post-operative radiotherapy on outcome following breast-conserving surgery

Trial	Number of patients	Tumour diameter (clinical)	Tumour excision	Regimen	Follow-up (years)	Local recurrence rates	Significance (*p*-value)
Milan III	579	<2.5 cm	2-3 cm grossly normal tissue	50 Gy plus 10 Gy boost to tumour bed	4.4	8.8% N Vs 0.3% R	0.001*
NSABP-B06	1843	< 4cm	1 cm gross & pathologi-cally clear	50 Gy No boost to tumour bed	9	43% N Vs 12% R	0.001*
Swedish	381	< 2 cm	2 cm pathologi-cally clear	54 Gy No boost to tumour bed	5	18.4% N Vs 2.3% R	0.001*
Scottish	585	< 4 cm	1 cm gross clearance	50 Gy 10-15 Gy boost to tumour bed	6	28.6% N Vs 6.2% R	
OCOG	799	< 4cm	Grossly and pathologi-cally clear	40 Gy and 12.5 Gy boost to tumour bed	5	30% N Vs 8% R	<0.0001*

R= Postoperative radiotherapy performed

N= No postoperative radiotherapy performed

Milan III: patients had quadrantectomy performed rather than a wide local excision

* Values reach significance

Adjuvant therapy

The use of adjuvant in breast cancer has been established for a numbers of decades. Adjuvant therapy usually entails either hormonal manipulation, commonly through drugs the like of tamoxifen, or is in the form of chemotheraputic agents. Adjuvant therapy has primarily been used to improve outcome. However there is increasing evidence that adjuvant therapy may effect local control. Early indication of this effect was witnessed when patients with breast cancer treated primarily by adjuvant therapy rather than surgery showed shrinkage of the tumour within the breast. These results subsequently lead to the use of "neo-adjuvant" chemotherapy in order to shrink inoperable tumour thus allowing surgery to be performed. Although this technique involves treating patients aggressively prior to obtaining formal histology from the tumour or an axillary nodal status, thus running the risks of over treatment, the results so far have been encouraging (90-106). Cunningham et al reviewed data from 167 patients with clinical stage IIB, IIIA or IIIB disease treated by neo-adjuvant chemotherapy (94). These patients were compared to 34 patients who received post-operative chemo-radiotherapy. Cunningham found that as well as allowing more limited surgery, neo-adjuvant therapy was associated with an improved overall survival (94). This difference did not however reach significance (94).

Furthermore, there was no difference in the local recurrence rates between these two groups of patients (94). This study however has limited numbers of patients, which may be inadequate in number to detect a small but significant difference. Ferriere et al analysed the data from a cohort of 329 patients who were considered to have inoperable tumours (106). In this study in 85 (26%) patients the use of neo-adjuvant therapy allowed tumour shrinkage sufficient to enable a breast-conserving procedure to be performed (106). In a further 80 (24%) patients there was complete clinical remission with these patients being subsequently treated by radiotherapy alone (106). However, an analysis of whether adjuvant therapy reduces local recurrence following surgery is more difficult to quantify. The effect of tumour-related characteristics as well as patient factors, all of which have a bearing on outcome, makes such studies difficult to perform.

Recent clinical trials suggest that the use of chemotherapy and tamoxifen either alone or in combination is associated with a reduction in both local recurrence and breast cancer-related death (107-113). However, in order to analyse any relationship between adjuvant therapy and local control studies containing large numbers of patients would be needed. There have however been two meta-analyses published that have analysed this specific subject (107-108). The "early breast cancer trialists' collaborative group" published

the results from a meta-analysis of the data from 75,000 women with breast cancer in 1992 (107). In a subgroup of 30,000 women results were available on the effect of tamoxifen on outcome (107). These patients were subdivided into those that were node positive or node negative (107). At 5-years those node negative patients receiving tamoxifen had an annual local recurrence rate of 3.62% compared to 5.22% among those not receiving tamoxifen (107). Similar results were found for the node positive cohort, 11.35% versus 15.29% (107). This difference in local control was only observed for the first 5 years after starting tamoxifen (107). Furthermore, although the presence of chemotherapy reduced the impact of tamoxifen's protective effect on local control, this effect still remained significant despite the use of chemotherapy (107).

The effect of chemotherapy on local control was also analysed in the meta-analysis by the "early breast cancer trialists' collaborative group." A total of 11,000 women in 31 trials were randomised to poly-chemotherapy (107). Data was also present on a further 8000 women from 16 separate trials randomised to receive single agent versus no chemotherapy (107). The use of chemotherapy was associated with a reduction of local recurrence (107). Due to the heterogeneity of the chemotheraputic regimes the authors of this trial subdivided the data in order to allow a more informative analysis to be

performed. When poly-chemotherapy regimes were compared with control the annual local recurrence rate fell from 15.12% to 10.96% (107). This protective effect against local recurrence was present among both node negative as well as node positive patients alike (107). This fall in local recurrence rate was only sustained for the first 5 years following surgery however (107).

The vast majority of poly-chemotheraputic regimens consisted of CMF. However, when regimes containing CMF in combination with other cytotoxic or hormonal agents were compared, the improvement in local control remained significantly better than among the control group of patients (107). Furthermore, the use of poly-chemotherapy appeared to better in preventing local recurrence than mono-chemotheraputic regimes (107). The duration of the chemotheraputic regimen also appeared to effect local control. The use of prolonged poly-chemotheraputic regimens appeared to be protective against local recurrence when compared to short term peri-operative regime (107).

This team also studied the impact of a range of immunotheraputic regimens on patient outcome. A total of 6300 women were randomised into 24 trials of immunotherapy (107). When performing the final analysis however the use of immunotherapy appeared to be associated with an increased local recurrence

rate (107). This was especially the case among those women receiving BCG vaccination who had a significantly poorer local control compared to controls (107).

Clahsen et al performed a meta-analysis analysing the effect of a short course of poly-chemotherapy on patient outcome (108). This team reviewed the data on 6093 patients from 5 clinical trials (108). When this short course of poly-chemotherapy was compared to controls, receiving chemotherapy resulted in a significant fall in the local recurrence rate (108). These results are in accordance with the early breast cancer trialists' collaborative group's results (107).

In summary the use of adjuvant therapy has been shown to be effective in helping reduce the incidence of local recurrence. This effect seems to be independent of axillary nodal status (107). However it is unclear whether such potentially toxic drugs should be used on node negative patients. This subject needs further evaluation.

EFFECT OF LOCAL RECURRENCE ON SURVIVAL

The effect of local recurrence on patient survival is a matter of intense debate. Furthermore it is difficult to determine any such correlation. The risk factors such as tumour size, lymphovascular invasion and tumour grade and type that predict for local recurrence are also those risk factors predictive of systemic recurrence. This aligned to the fact that when considering the effect of local on systemic recurrence the impact of lead-time bias has to be taken into account makes any study setting out to determine such a correlation extremely difficult to design. Data collected from the NSABP-B06 trial has shown marked differences in the rates of local recurrence rates between those offered post-operative radiotherapy and those who were not (38). However, despite these differences in local recurrence rates, there was no significant difference in overall survival among the various treatment arms of this trial (38). This has led to the suggestion that there is no association between local recurrence and distant disease (114).

However, when patients who suffer a local recurrence are analysed as a separate population and outcomes assessed, it has been noted that the relative risk of developing systemic disease increases to between 1.2 to 6.6 (32,38). In the NSABP-B06 trial when a time dependent multivariate analysis was

performed the presence of local recurrence was found to be a predictor of systemic recurrence, relative risk of 3.41 (38). Veronesi et al found similar results when they reviewed their experience of 2233 patients (32). In the NSABP-B06 trial it was shown that patients with local recurrence occurring within 1 year of the initial surgery had a higher incidence of systemic recurrence (38). Similarly, Veronesi showed the relative risk of developing systemic disease to be 6.6 if the local recurrence had occurred within one year of surgery (32). This risk fell to 2.2 and then to 1.2 for local recurrences occurring firstly 2 and then 3 years following surgery (32). Furthermore, analysis of results of the OCOG trial has shown that local recurrence is associated with poorer survival, relative risk being 2.18 (62). Similarly, Chauvet et al showed that the 5-year survival was 87.5% for those with local recurrence compared to 91.3% for those without (115). These results have been repeated in other studies (31,116-126).

Voogd et al analysed the pattern of relapse from the BORST trial and published results on 266 patients with local recurrence (119). This team found that patients with local recurrence suffered from poorer survival (119). However, the clinico-pathological characteristics of the local recurrence were also found to be predictors of poorer outcome. Patients presenting with palpable rather than mammographically detected recurrences had poorer

survival (119). This finding reflects the fact that patients with larger recurrences have poorer outcomes in terms of survival (119). Furthermore, not surprisingly those patients with recurrences that were invasive rather than in-situ had a poorer survival (119).

Despite this wealth of data the numbers of local recurrences seen in each of the above studies is relatively small. Furthermore, all the above studies have taken place over a prolonged period of time during which there have invariably been changes in adjuvant therapy protocols offered to patients. Such changes in themselves can have an impact on both local and systemic recurrence. Such variables have a detrimental effect on the power of the above studies to adequately assess any correlation between local and systemic recurrence. One possible method of counteracting these problems would be through the use of a meta-analysis technique.

In summary although breast-conserving surgery has gained in popularity the concerns about local recurrence and its effect on patient outcome remain. As yet although there is a wealth of evidence correlating local recurrence with poorer outcome, all these studies contain relatively small numbers of patients with local recurrence making such results difficult to interpret. Furthermore, none of the above studies have been able to explain the relationship between

local failure and distal disease. One possibility is that local recurrence is an indicator of an aggressive disease process. The other possibility is that local recurrence causes systemic disease. In order to support a causal relationship between local and systemic recurrence there would be the need to demonstrate that by reducing the local recurrence rate a fall in systemic recurrence also occurs. To date there have been published several randomised trials demonstrating that post-operative radiotherapy results in a low local recurrence rate (17,38,62-64,76-80). However, in these trials there was no detected difference in overall survival among those patients not receiving radiotherapy. Despite the large numbers of patients included within these individual trials the numbers included may not be sufficient however to detect small difference in survival. In the meta-analysis by the "early breast cancer trialists' collaborative group" however a fall in the breast cancer-related death rates among those receiving radiotherapy was seen (88). This finding suggests that there may well be a direct causal relationship between local and systemic recurrence (88). Despite this, as inadequate tumour excision is a major risk factor for the development of local recurrence, the importance of an adequate primary operation cannot be overestimated.

MULTICENTRICITY OF BREAST CANCER

Several studies have shown that significant proportion of patients with breast cancer have multicentric disease (127-154). Vaidya et al showed that multicentric disease was present in 63% of patients (127). Furthermore, although the majority of primary tumours within this trial occurred in the upper outer quadrant of the breast, multicentric foci were distributed evenly throughout all quadrants of the breast (127). This group calculated that following a wide local excision of the primary tumour in 60% of cases residual multicentric foci would not have been excised (127). Furthermore, several researchers have found that in 50-62% of cases multicentric disease is genotypically different from the primary tumour (155-156). Other groups have found that histologically normal lobules surrounding the primary tumour express the same genotype as the cancer (157). All these results suggest that a significant proportion of patients undergoing breast-conserving surgery show a field change within the breast.

However, the majority of local recurrences occur close to the site of the previous operation or within the same quadrant as the primary (54). Thus, although the presence of multicentric disease within the breast is well recognised, it's clinical significance remains uncertain. Megee et al showed

that when radiotherapy to the whole breast was replaced by irradiation to the site of the tumour alone, the local recurrence rate rose from 13 to 25% (158). However, on closer inspection of this data, the increase in the local recurrence rate only occurred among patients with lobular carcinoma (158). Patients with invasive ductal carcinoma however showed a non-significant increase in the local recurrence rate from 11 to 15% (158).

In summary, although local recurrence is associated with inadequate tumour excision, the exact mechanism controlling the development of local recurrence is not completely understood.

SUMMARY

Over the past decade breast-conserving surgery has become increasingly popular. Local recurrence however remains a continuing problem however. Inadequate tumour excision has been shown to be one of the major risk factors for the development of local recurrence. Inking and tumour bed assessments are the two main techniques used to assess the adequacy of tumour excision. Previous studies have shown that tumour bed assessment is superior to inking in assessing the presence of residual disease.

The following thesis is an analysis of the experience of breast-conserving surgery at one institution over a period of 13 years. The majority of patients had tumour bed assessment performed. Six specific issues will be investigated:

1) Incidence of tumour bed positivity.

2) Correlation between clinico-pathological factors and tumour bed positivity.

3) Impact of tumour bed positivity on patient outcome.

4) Effect on the local recurrence rate of implementing a pragmatic policy of tumour bed assessment combined with selective re-excision.

5) Correlation between pre-operative mammographic features and tumour bed positivity.

6) Correlation between tumour bed positivity and occult axillary disease.

The aim of this thesis is to expand upon the current knowledge in these specific areas in order to have a beneficial impact on clinical practice.

CHAPTER 1

INTRODUCTION

With the advent of radiotherapy surgical treatment of primary operable breast cancer by the use of breast-conserving surgery has become increasingly popular. A number of factors have influenced this change, the most important of which has been the publication of several clinical trials in the late 1980's and early 1990's that showed no survival advantage of mastectomy over breast-conserving surgery (15,16,31,38). The two major trials conducted over this period were by Veronesi in Milan and Fisher from the United States (15,38). Veronesi et al published results on 701 women between 1971 and 1980 with primary tumours of 2cm or less (15). In this trial conservation surgery consisted of a quadrantectomy and a level 3 axillary dissection (15). This Milan trial found no survival advantage of mastectomy over breast-conserving surgery (15). Fisher et al published the 10-year results of the NSABP-B06 trial in 1991 (38). This team also found that the distant disease-free survival for those patients undergoing mastectomy was comparable to those undergoing a breast-conserving procedure (38).

The decision to offer breast-conserving surgery depends on several factors including tumour size; site and patient preference. The aim of breast-conserving

surgery lies in providing an excellent cosmetic result while achieving local control. Local tumour recurrence following this form of surgery however remains a problem. Several studies have showed that local recurrence rates vary from 0.3 to 15% (17,38,62-64). There has been much debate about the impact of local recurrence on patient survival. Results from the NSABP-B06 trial have shown that although there was no difference in survival between those patients undergoing a mastectomy compared to breast-conserving surgery, among those patients who went on to develop local recurrence there was a strong association with subsequent systemic disease, relative risk of 3.41 (38). Similarly results from the OCOG trial randomising 837 patients to radiotherapy following initial breast-conserving surgery found that local recurrence was associated with an increased risk of subsequent systemic recurrence, relative risk of 2.11 (62). Thus although breast-conserving surgery has become widely accepted, the importance of achieving a low local recurrence rate cannot be underestimated.

The literature is awash with analyses of the risk factors for local recurrence following breast-conserving surgery (17,32,38,62-64,76-80). Recognised risk factors include poorer tumour grade; extensive in-situ disease within the tumour and lymphovascular invasion. The major risk factor for developing local recurrence however is inadequate tumour excision. Due to the fact that patients with a positive margin invariably undergo further surgery, a direct association between positive margins and local recurrence has been difficult to identify in

the published literature. However the study by Holland et al does provide indirect evidence of a link between positive margins and local recurrence (33). In this study a total of 282 mastectomy specimens from patients thought to be suitable for breast-conserving surgery were sectioned (33). Holland et al found that within a 2cm rim of grossly normal tissue surrounding the tumour, residual disease was present in 43% of cases (33). This figure is similar to the local recurrence rate seen in the NSABP-B06 trial among patients not receiving post-operative radiotherapy (38). A number of different factors make the completeness of surgical excision difficult to determine however. Firstly lumpectomy specimens usually have a large and irregular surface area, containing a non-uniform tumour, thus making analysis difficult. Furthermore, the pathologist commonly has to deal with specimens containing eccentric intraductal tumour extension (159). All these problems make gross assessment of tumour margin unreliable.

Attempts have been made to determine the adequacy of tumour clearance more precisely. The two methods commonly used to determine tumour clearance are India ink staining of the lumpectomy specimen and pathological assessment of shavings from the residual cavity following initial wide local excision. Assessment of the tumour margin using the India ink staining involves the pathologist dipping the specimen in ink followed by taking random biopsies from the surface of the specimen in order to detect whether the tumour lies

"close" to or actually crosses this inked margin. This technique of tumour margin assessment was used in both the Milan and the NSABP-B06 trials (17,38). Unfortunately within the literature there is no consensus regarding a positive inked margin. Typically margin involvement is found in 10% of cases (38). However if Holland's results are taken into account then this figure underestimates the extent of residual disease (33). Furthermore, Frazier et al found that in patients undergoing a re-excision after a negative inked margin residual disease was present in 30% of cases (160). Thus the main concern about the technique of inking is that it may be underestimating the incidence of positive margins.

An alternative approach to the problem of assessing completeness of excision is to analyse what is left in the breast rather than what is at the surface of the excised specimen. The technique of cavity wall shaving has previously been described (61). Macmillan et al reviewed 264 patients undergoing breast-conserving surgery, all of whom had tumour bed assessment performed (61). In this study the incidence of residual disease within the tumour bed was 39.3% (61). In 51.5% of cases this disease was purely in-situ in nature (61). This institution performed a policy of selective surgery, either mastectomy or re-excision, for those patients with positive cavity shavings. Of the patients with a positive margin, 36 (35.6%) underwent further surgery of whom 50% had evidence of residual disease (61). This study does however have several

limitations. Firstly due to the retrospective nature of the study the cohort of patients is relatively small and non-randomised. This weakens any subsequent statistical analysis. Furthermore the policy of this unit about proceeding for further surgery does not seem to be uniform in nature. Of the patients with a positive margin only 35.6% underwent further surgery. This may well reflect a change in clinical practice over the period of this study, however not comment on this fact is made by the authors. In order analyse the efficacy of tumour bed assessment in detecting residual disease as well as having an impact on lowering the local recurrence rate a much larger study with longer follow-up would be needed.

Beck et al performed a prospective study on 144 patients directly comparing cavity shavings with inking of margins (162). In this study all patients underwent a wide local excision followed by biopsies of the superior, inferior, medial and lateral walls of the residual cavity. Following this, formal cavity shavings were performed (162). The lumpectomy specimen was subsequently fixed and inked (162). Beck et al found that in 43% of cases there was evidence of a positive margin detected by all three methods (162). Both tumour bed assessment and inking detected a positive margin in 27% of the cases compared to only 17% for bed biopsy (162). The incidence of positive bed biopsy seen in this study seems to be less than that of 25% reported in the literature however (163). Interestingly although both inking and cavity shavings detected a

positive margin in 27% of cases, in only 63% of these cases was there concordance between both these techniques (162). This suggests that the presence of a positive inked margin is a poor predictor of residual disease as assessed by cavity shavings. The finding of a clear inked margin in the presence of disease within the cavity shavings is of clinical concern. This discrepancy may be explained by the fact that tumour bed assessment as well as detecting residual disease also detects foci of disease distant from the primary and thus has an inherently better detection rate than the technique of inking.

In summary breast-conserving surgery has been shown to be a safe alternative to mastectomy. However, local recurrence remains a continuing problem. One of the major risk factors for the development of local recurrence is inadequate tumour excision. Several techniques to assess the tumour margin have been developed. The use of tumour bed assessment appears to detect a higher incidence of positive margins compared to inking. The aim of this study is to expand upon previous work correlating clinico-pathological factors that predict for tumour bed positivity as well as ascertaining the overall incidence of tumour bed positivity. Furthermore, the effect of tumour bed positivity on patient outcome will also be analysed.

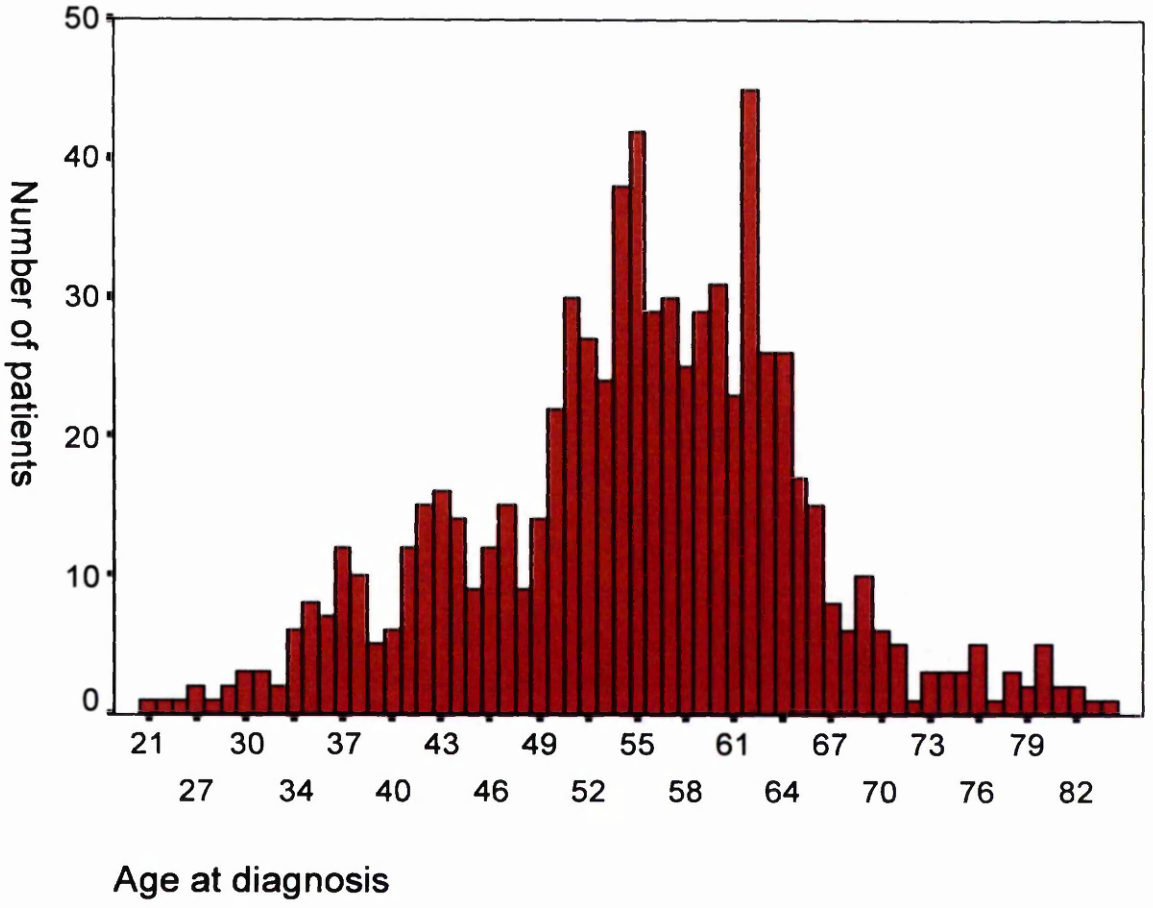
PATIENTS AND METHODS

Patients

A total of 752 patients entered the study between September 1982 and December 1995. All had stage I or II breast cancer. Of these, a total of 543 (72%) patients had cavity shavings performed following initial wide local excision. Patients in whom cavity shavings were not performed were operated upon prior to December 1987. The mean age of patient was 54.88 years (range 21-84, S.D. 10.38 years, Graph 1.1).

Four hundred and eighty four (64%) patients presented symptomatically and 112 (15%) patients had screen-detected palpable tumours, while 156 (21%) patients had screen-detected impalpable tumours. Symptomatic patients presenting to out-patient clinics had a preoperative diagnosis made by a combination of clinical assessment, mammography (both cranio-caudal and oblique views performed) and fine needle aspiration (FNA) cytology and/or tru-cut biopsy.

Graph 1.1: Distribution of patients' age at diagnosis



Surgical technique

All patients underwent a wide local excision with a 1-2cm macroscopic clearance. The deep margin of excision was the pectoralis fascia. Wherever necessary, a skin ellipse overlying the tumour was excised encompassing the fine needle aspiration/tru-cut biopsy site or areas of skin tethering. With the introduction of tumour bed assessment from 1988 onwards, following initial wide local excision, tissue forceps were applied to the wall of the residual cavity and a thin shaving of tissue was excised (cavity shavings)(Illustration 1.1). This tissue was submitted separately for histopathological analysis.

Of patients that attended via the National Health Service Breast Screening Programme (NHSBSP) with impalpable lesions, if a pre-operative core biopsy had been obtained by ultrasound or stereotaxis, definitive surgery was subsequently performed. Otherwise, a diagnostic biopsy was followed by definitive surgery. In all, 85% of patients had a level II axillary clearance performed.

Illustration 1.1: Technique of performing cavity shavings

Pathological assessment

All tissue submitted was assessed in the pathology department of the Western Infirmary, Glasgow and assessed by a pathologist specialising in breast disease. The maximum tumour and lumpectomy diameters as well as tumour grade (Bloom and Richardson) were recorded (163). The presence of an intraduct component within the tumour was also noted and classified as being absent, minimal or extensive in nature. Where commented on by the pathologist, the presence of lymphovascular invasion, neural invasion or a lymphocyte infiltrate surrounding the tumour were also recorded. Tissue was also analysed for oestrogen receptor status. This analysis was initially performed biochemically, however from the early 1990's the use of immunohistochemical techniques were increasingly applied. Thus for the purposes of this study the oestrogen receptor status was recorded as being positive or negative rather than as a continuous variable.

The cavity shavings were blocked, sectioned and examined for the presence of invasive and/or in-situ disease. The total number of blocks of tissue processed from the cavity shavings varied from 4 to 54. The number of foci of disease within the cavity shavings, as well as the number of blocks involved with disease, were also recorded.

Adjuvant therapy

In general, patients who had axillary nodal disease received adjuvant systemic therapy. This was in the form of tamoxifen and chemotherapy, either given alone or in combination. If patients were oestrogen receptor positive they were offered tamoxifen at the dose of 20mg per day. If however, they fell into a poor prognostic category as assessed by the Nottingham Prognostic Index (164) they were offered chemotherapy consisting in the majority of cases of 6 pulses of cyclophosphamide, methotrexate and 5-flurouracil. A total of 529 (70.3%) patients received tamoxifen and 98 (13%) patients received adjuvant chemotherapy. In all, 551 (73.3%) patients received post-operative radiotherapy to the breast consisting of 46 Gy in 23 fractions over 35 days with a 12 Gy boost to the tumour bed in 4 fractions over 7 days. Of the 201 patients not receiving adjuvant radiotherapy, in 57 (28.3%) cases this was due to the fact that these patients went on to have a mastectomy following initial attempts at breast-conserving surgery. The remaining 144 (71.7%) of these patients were those entered into clinical trials and randomised to not receive radiotherapy.

Statistical analysis

Statistical analysis was performed using a SPSS (version 7.51) statistics package. This allowed the calculation of simple frequencies and percentages from the available data. More complex statistical analyses were needed in order to correlate tumour bed positivity with established clinico-pathological prognostic factors. In this situation a Pearson's chi-squared test was used to calculate significance. Factors assessed included mode of presentation as well as tumour pathological factors. The Pearson's chi-squared test was also used to compare the sociological distribution of this cohort of patients with the national average. A comparison of mode of presentation as well as the Nottingham Prognostic Index (164) with deprivation category was also performed. When a comparison of means was required the Independent sample **t**-test was used. This was used to compare patient age with tumour bed positivity. A Mann-Whitney test was used to compare lumpectomy and tumour diameters with tumour bed positivity. In order to perform a multivariate analysis of the factors that predict for tumour bed positivity a stepwise Logistic Regression analysis was performed. A 5% significance level was used throughout this analysis.

In order to assess the effect of cavity shaving positivity on patient outcome, at the end of the period of follow-up, three measures of outcome were assessed:

1: Disease-free survival (DFS): defined as those patients who are alive and well and not having sustained a local recurrence, contralateral breast tumour, axillary recurrence or systemic recurrence.

2: Distant disease-free survival (DDFS): defined as those patients being free of systemic disease and not dying from breast cancer.

3: Overall survival (OS): defined as those patients not dying from breast cancer.

Survival curves were calculated using the Kaplan-Meier technique. In order to compare the effect of tumour bed positivity on DFS, DDFS and OS the log-rank test was performed. The log-rank test was also used to analyse any relationship between tumour bed positivity and local recurrence. Multivariate analyses using the Cox proportional hazard model (165) were used to produce a set of in-depth useful predictions of survival. The model was performed in a stepwise manner with only the co-variates reaching a 5% significance level being included in the final model. The co-variates included in the model were age at diagnosis, maximum tumour diameter (pathological), tumour grade, axillary nodal status and oestrogen receptor status.

RESULTS

Socio-economic grouping

The Western Infirmary, Glasgow is a tertiary referral centre for breast cancer in the West of Scotland. This is reflected in the patient population, where 47.8% of the 575 of patients on whom postcode information was available, lived out side Glasgow.

In order to gain a better understanding of the socio-economic distribution of this group of patients we correlated postcode data with deprivation category information supplied by Glasgow University.* A breakdown of these results and comparison to the expected national average is represented in Table 1.1. As can be seen from these results, there is a greater than expected representation from deprivation category ranges 1-2 and 6-7 among the Western Infirmary patients than the expected national average. A Pearson's chi-squared test however showed no statistically significant difference between the two populations, p=0.099.

Deprivation category data was also compared with mode of presentation. A similar analysis as to the above was performed. When patients in deprivation categories 1-2 and 6-7 were compared although there were differences between

observed and expected outcomes as for mode of presentation, these were not statistically significant (Table 1.2). The Nottingham Prognostic Index (164), which was calculated on 570 patients, was compared with deprivation category data. When a Pearson's chi-squared test was performed there was no significant association between poorer deprivation category and a poorer Nottingham Prognostic Index.

* Deprivation category is used by sociologists in order to categorise people living in certain postcode areas of the country into various socio-economic groups. Deprivation category 1 or 2 refers to people living in affluent areas where as categories 6 or 7 refers to deprived areas of the country.

Table 1.1: Deprivation category

Deprivation category range	Western Infirmary data (%)	Expected Scottish average (%)
1-2	30.3	24.2
3-5	45.2	59.7
6-7	24.5	16.1

Comparison between Western Infirmary patients and the Scottish average as for deprivation category ranges.

Table 1.2: Presentation versus deprivation category

Deprivation category	Symptomatic presentation		Screen-detected (impalpable tumour)	
	Observed count	Expected count	Observed count	Expected count
1-2	91	94.5	44	40.5
6-7	94	81	27	35

Comparison of mode of presentation, either symptomatic or screen-detected non-palpable tumours, with deprivation category range.

Operative pathology

In all, 631 (84%) patients presented with invasive ductal carcinoma, whereas 49 (6.5%) patients had lobular carcinoma and 72 (9.5%) patients presented with tumours of special type. The mean tumour diameter was 13.79 mm. Furthermore, the mean tumour diameter for symptomatic patients was 16.10mm compared to 13.19mm for those patients with screen-detected yet palpable tumours, $p<0.0001$.

A level II axillary clearance was performed on 643 (85%) patients. In 617 of these patients, data on number of positive nodes as well as the total number of nodes retrieved was available. Information on axillary node positivity is presented in Table 1.3. The mean number of nodes retrieved was 11 (range 2-30) with a total of 179 (29%) of these patients being node-positive.

Table 1.3: Lymph node positivity

	Number of cases	**Percentage**
Node negative	438	**71**
1-3	136	**22**
4-10	34	**5.5**
>10	9	**1.5**
Total number of cases	617	**100**

Tumour bed positivity

Cavity shavings were performed in order to ascertain tumour margin clearance in a total of 543 (72%) patients. Based on the type and extent of disease, a proportion of patients underwent further surgery - either re-excision or mastectomy. The decision to offer further surgical intervention was a pragmatic one and depended on the type and extent of disease. Patients with extensive invasive and/or in-situ disease involving most of the cavity shavings underwent a mastectomy. Those patients with minimal to moderate invasive and/or in-situ disease - for example 1 focus of invasive disease in one block combined with in-situ disease within three to four blocks had a re-excision performed. In those patients with very minimal disease – for example one to three foci of in-situ disease limited to one block had no further surgery (Illustration 1.2).

A total of 200 (37%) patients were tumour bed positive. Of these, 26 (13%) patients had a re-excision performed. When the re-excised tissue was examined histologically, 11 (37.9%) patients had residual invasive or in-situ disease. In a further 57 (29%) patients a mastectomy was performed. In this subgroup of patients, 17 (30%) patients had residual invasive or in-situ disease within the mastectomy specimen. Of the 117 (58%) patients who were tumour bed positive but did not undergo further surgery all had between 1 to 3 foci of

disease confined to one block of tissue. Patients who underwent mastectomy were subsequently excluded from the majority of the following analyses.

Of the 486 breast-conserved patients a total of 143 (29.4%) patients had residual disease in the cavity shavings, Table 1.4. The presence of disease varied from a single focus of DCIS in 1 block to invasive disease present within several blocks.

Although the total number of blocks of tissue processed from the cavity shavings varied from 4 to 54, there was no association between tumour bed positivity and the number of blocks processed from the cavity shavings, p=0.075.

Illustration 1.2: Flow chart representing further surgical intervention

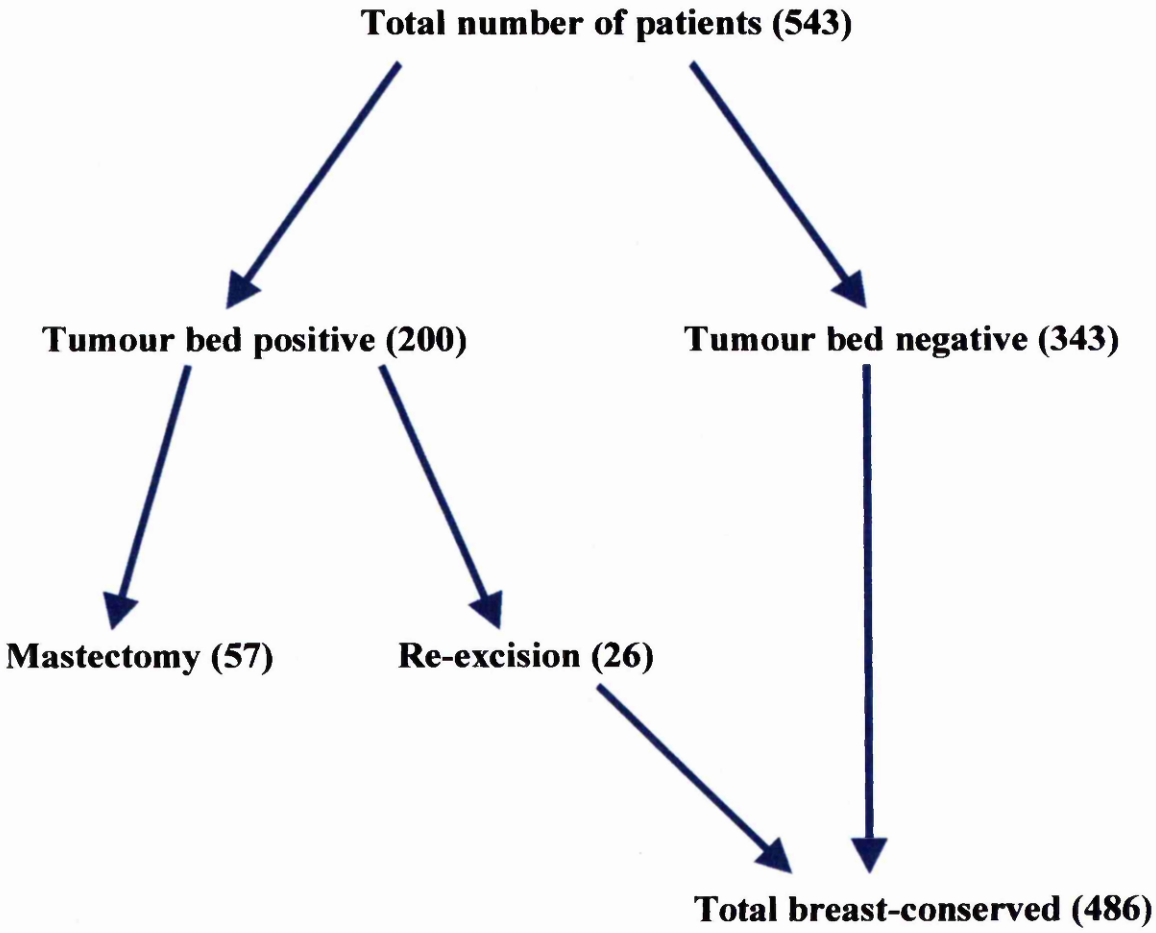


Table 1.4: Incidence of tumour bed positivity

Type of disease present	Number of cases	Percentage of total number of cases
Invasive carcinoma (or mixed invasive/in-situ disease)	51	10.5
In-situ disease	92	18.9
Total positive	143	29.4
Total negative	343	70.6
Total number of cases	486	100

Effect of patient age on tumour bed positivity

The relationship between patient age at diagnosis and tumour bed positivity was examined using an independent sample **t**-test. The mean age of tumour bed positive patients was 54 years compared to 56 years for tumour bed negative patients, p=0.064.

Effect of clinical presentation

The relationship between mode of clinical presentation and the incidence as well as type of disease within the cavity shavings is represented in Tables 1.5-1.6. Patients presenting symptomatically had an increased incidence of tumour bed positivity when compared with screen-detected patients, p=0.042.

Table 1.5: Clinical presentation and tumour bed positivity

Mode of presentation	Cavity shavings positive	Cavity shavings negative	Total number of cases
Symptomatic	91 **(33)***	184 **(67)**	275
Screen-detected palpable tumours	14 **(18)**	62 **(82)**	76
Screen-detected impalpable tumours	38 **(28)**	97 **(72)**	135

* p=0.042

Table 1.6: Clinical presentation and type of tumour bed disease

Mode of presentation	Invasive (or mixed invasive/in-situ disease)	In-situ disease	Total number of cases
Symptomatic	35 **(38)**	56 **(62)**	91
Screen-detected palpable tumours	4 **(29)**	10 **(71)**	14
Screen-detected impalpable tumours	12 **(32)**	26 **(68)**	38

- Values in parentheses are percentages

Effect of extent of excision

Tumour diameter ranged from 5 to 35 mm (mean 13.55mm, S.D. 6.60mm). The mean tumour diameter for tumour bed positive patients was 14.41mm compared to 13.20mm for tumour bed negative patients, p=0.068. Furthermore, the mean lumpectomy diameter for tumour bed positive patients was 59mm compared with 63mm for tumour bed negative patients, p=0.075.

Overall, 468 patients had the tumour diameter recorded. In order to assess the effect of both tumour diameter and lumpectomy diameter on tumour bed positivity, the lumpectomy diameter was tabulated against cavity shaving status for a range of tumour diameters. The Mann-Whitney test was used to detect any underlying associations. These results are represented in Table 1.7. For patients with tumour diameters of 21-30mm, the mean lumpectomy diameter of the tumour bed positive group was significantly smaller than the tumour bed negative group, p=0.029.

Table 1.7: Relationship between tumour/ lumpectomy diameter and tumour bed positivity

	Mean lumpectomy diameter (mm)			
Tumour diameter	**Cavity shavings positive**	**Cavity shavings negative**	**Total number of cases**	**Significance (*p*-value)**
<10mm	55.7	59.9	199	0.052
11-20mm	61.6	64	228	0.691
21-30mm	65.6	76.5	37	**0.029**
>30mm	0	83.7	4	Not calculable

Effect of tumour pathological factors

The relationship between tumour pathological factors and tumour bed positivity is represented in Tables 1.8-1.15. Tumour bed positivity was significantly associated with tumour grade II, p=0.0001. When the Mental-Haenszel chi-squared test was performed there was an association between poorer tumour grade and tumour bed positivity, p=0.042. The presence of lymphovascular invasion was associated with the presence of invasive disease within the cavity shavings,

p=0.021. An extensive in-situ component within the tumour was associated with both tumour bed positivity and the presence of in-situ disease within the cavity shavings, p=0.002 and 0.010 respectively. Conversely, oestrogen receptor positivity was found to be associated with tumour bed positivity, p=0.045.

Effect of axillary nodal status

The relationship between tumour bed positivity and axillary nodal disease is represented in Tables 1.16-1.17. There was a significant relationship between invasive (or mixed invasive/ in-situ) disease in the cavity shavings and axillary nodal disease, p=0.009. There was also a trend towards the presence of invasive disease within the cavity shavings and an increasing number of positive nodes, p=0.007.

Table 1.8: Tumour grade and tumour bed positivity

Tumour grade	Cavity shavings positive	Cavity shavings negative	Total number of cases
Grade I	21 **(20)**	84 **(80)**	105
Grade II	92 **(42)***	125 **(58)**	217
Grade III	14 **(19)**	61 **(81)**	75
Not defined	16 **(18)**	73 **(82)**	89

* p=0.0001

Table 1.9: Tumour grade and type of tumour bed disease

Tumour grade	Invasive (or mixed invasive/in-situ disease)	In-situ disease	Total number of cases
Grade I	8 **(38)**	13 **(62)**	21
Grade II	28 **(30)**	64 **(70)**	92
Grade III	6 **(43)**	8 **(57)**	14
Not defined	9 **(56)**	7 **(44)**	16

- Values in parentheses are percentages

Table 1.10: Lymphovascular invasion and tumour bed positivity

	Lymphovascular invasion present	Total number of cases
Cavity shavings positive	25 **(17)**	143
Cavity shavings negative	53 **(15)**	343

Table 1.11: Lymphovascular invasion and type of tumour bed disease

	Invasive (or mixed invasive/in-situ disease)	In-situ disease	Total number of cases
Lymphovaseular invasion present	14 **(56)***	11 **(44)**	25

* p=0.021

- Values in parentheses are percentages

Table 1.12: Tumour in-situ component and tumour bed positivity

Type of in-situ disease within tumour	Cavity shavings positive	Cavity shavings negative	Total number of cases
Extensive in-situ disease	90 **(36)***	158 **(64)**	248
Minimal in-situ disease	20 **(27)**	53 **(73)**	73
Absence of in-situ disease	33 **(20)**	132 **(80)**	165

* p=0.002

Table 1.13: Tumour in-situ component and type of tumour bed disease

Type of in-situ disease within tumour	Invasive (or mixed invasive/in-situ disease)	In-situ disease	Total number of cases
Extensive in-situ disease	28 **(31)**	62 **(69)***	90
Minimal in-situ disease	8 **(40)**	12 **(60)**	20
Absence of in-situ disease	15 **(45)**	18 **(55)**	33

* p=0.01

- Values in parentheses are percentages

Table 1.14: Oestrogen receptor status and tumour bed positivity

	Cavity shavings positive	Cavity shavings negative	Total number of cases
Oestrogen receptor positive	104 **(32)***	225 **(68)**	329
Oestrogen receptor negative	25 **(22)**	90 **(78)**	115

* p=0.045

Table 1.15: Oestrogen receptor status and type of tumour bed disease

	Invasive (or mixed invasive/in-situ disease)	In-situ disease	Total number of cases
Oestrogen receptor positive	38 **(37)**	66 **(63)**	104
Oestrogen receptor negative	11 **(44)**	14 **(56)**	25

- Values in parentheses are percentages

Table 1.16: Nodal status and tumour bed positivity

Axillary nodal status	Cavity shavings positive	Cavity shavings negative	Total number of cases
Node negative	89 **(27)**	235 **(73)**	324
1-3 nodes positive	30 **(36)**	53 **(64)**	83
> 3 nodes positive	6 **(33)**	12 **(67)**	18

Table 1.17: Nodal status and tumour bed disease

Axillary nodal status	Invasive (or mixed invasive/in-situ disease)	In-situ disease	Total number of cases
Node negative	26 **(29)**	63 **(71)**	89
Node positive	19 **(53)***	17 **(47)**	36
1-3 nodes positive	15 **(50)**	15 **(50)**	30
> 3 nodes positive	4 **(67)**	2 **(33)**	6

* p=0.009

- Values in parentheses are percentages

Multivariate analysis of predictors of tumour bed positivity

A multivariate analysis of all the factors which predicted for tumour bed positivity was performed, Table 1.18. On performing this analysis only poorer tumour grade; extensive in-situ disease within the tumour and axillary nodal disease predicted for tumour bed positivity.

Table 1.18: Multivariate analysis of predictors of tumour bed positivity

Factor	Significance (*p*-value)	Relative risk	95% Confidence interval
Symptomatic presentation	0.2078	0.7627	0.4724-1.2314
Oestrogen receptor positivity	0.0505	0.6068	0.3678-1.0012
Lymphovasular invasion	0.1178	0.6975	0.4441-1.0955
Poorer tumour grade	**0.0457***	2.5992	1.0186-6.6327
Axillary nodal disease	**0.0002**	0.4390	0.2838-0.6791
Extensive in-situ disease within the tumour	**0.0236**	0.5952	0.3798-0.9328

* Comparing grade II and III with grade I patients.

Effect of tumour bed positivity on patient outcome

Patients were followed-up at a 3 monthly interval at outpatient clinics during the first 5 years following surgery and then at 6 monthly intervals thereafter. The mean follow-up was 52 months (range 14-105 months). Patient outcomes are presented in Table 1.19.

Table 1.19: Patient outcomes

Patient outcomes	Cavity shavings positive	Cavity shavings negative
Local recurrence	4 **(2.8)**	7 **(2)**
Axillary recurrence	5 **(3.5)**	1 **(0.3)**
Contralateral breast cancer	3 **(2)**	4 **(1)**
Alive with systemic recurrence	6 **(4)**	6 **(1.7)**
Breast cancer-related death	13 **(9)**	16 **(4.7)**
Non-breast cancer-related death	15 **(10.5)**	5 **(1.5)**
Total number of cases	143	343

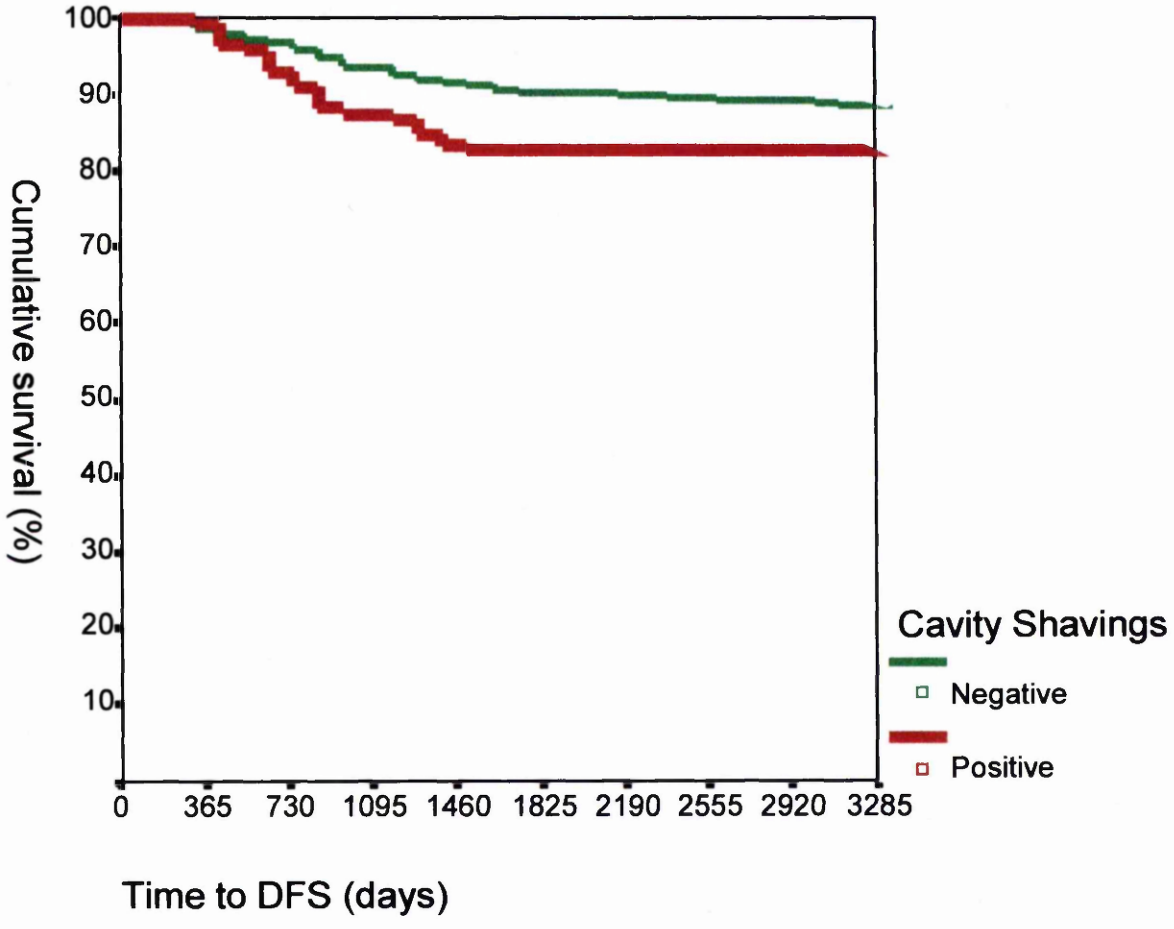
- Values in parentheses are percentages

Probability of Disease-Free Survival (DFS)

The linear representation of DFS for the cavity shavings positive and negative cohorts is shown in Graph 1.2. On performing the log-rank test, tumour bed positive patients had a poorer DFS, p=0.0445. When the effect of tumour bed positivity on local recurrence was examined however, there was no evidence of an association between disease within the tumour bed and local recurrence, p=0.7941 (log-rank test).

On performing a Cox regression analysis of all the co-variates, only oestrogen receptor negativity and axillary nodal disease predicted for poorer DFS, p=0.0062 and 0.0061 respectively (Table 1.20). These co-variates were then entered into the final model which demonstrated that the presence of disease within the cavity shavings was a predictor of poorer DFS, p=0.0140 (relative risk 2.0803, 95% confidence interval 1.1595-3.7325).

Graph 1.2: Linear representation of Disease-Free Survival (DFS)



* p=0.0445

Numbers of patients at risk	Time Zero	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year	9th year
Cavity Shaving negative	343	338	274	232	181	119	79	45	24	2
Cavity Shaving positive	143	140	113	98	76	55	32	23	11	5

Table 1.20: Cox regression analysis of Disease-Free Survival (DFS)

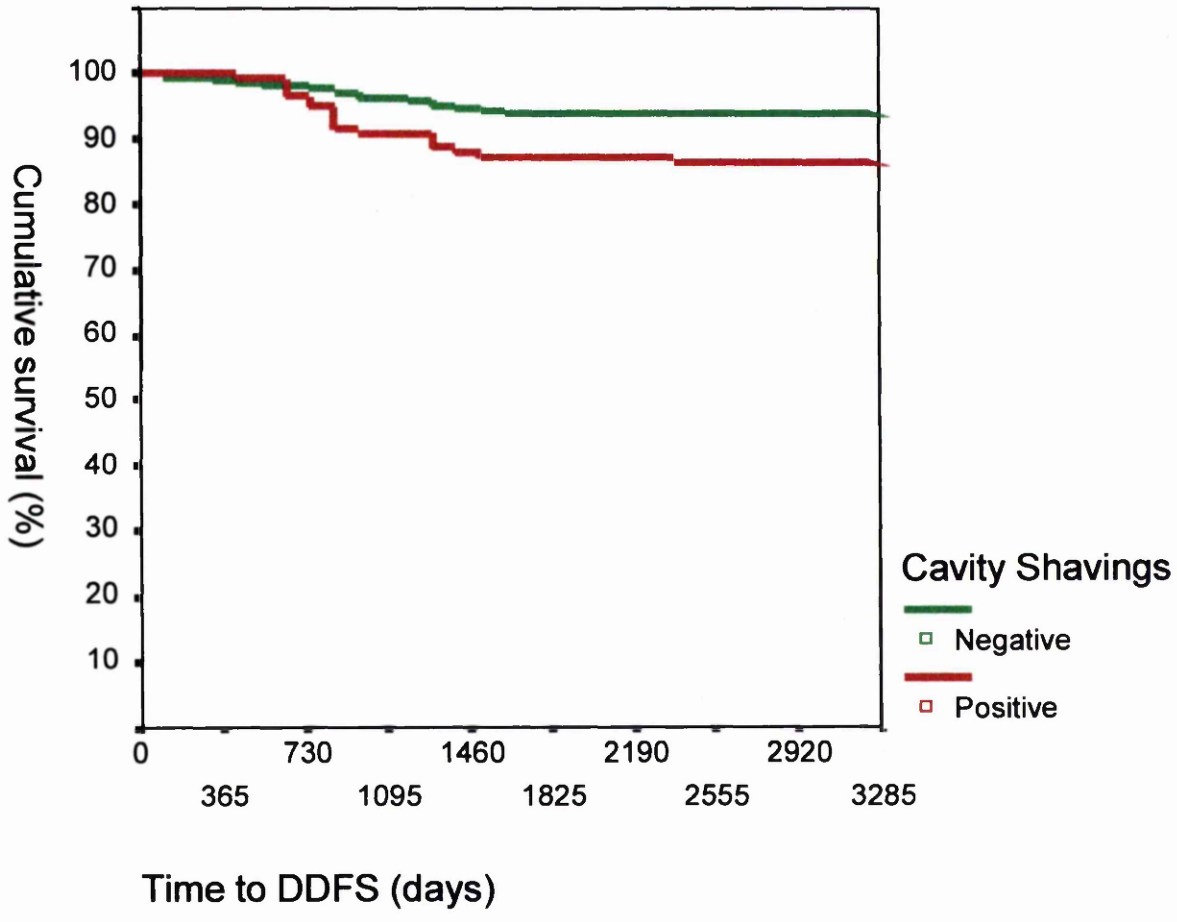
Co-variate	Significance (*p*-value)	Relative risk	95% Confidence interval
Age at diagnosis	0.3811	0.9879	0.9614-1.0151
Maximum tumour diameter	0.4808	1.0161	0.9719-1.0624
Poorer tumour grade	0.1142	1.2869	0.9411-1.7597
Axillary nodal disease	**0.0061**	1.6135	0.9769-1.0669
Oestrogen receptor negativity	**0.0062**	2.4065	1.2834-4.5125
Tumour bed positivity	**0.0140**	2.0803	1.1595-3.7325

Probability of Distant Disease-Free Survival (DDFS)

The linear representation of DDFS for the cavity shavings positive and negative cohorts is shown in Graph 1.3. On performing the log-rank test, the tumour bed positive patients had a poorer DDFS, p=0.0092.

On performing a Cox regression analysis of all the co-variates, only oestrogen receptor negativity and axillary nodal disease predicted for poorer DDFS, p=0.0003 and 0.0118 respectively (Table 1.21). These co-variates were then entered into the final model which demonstrated that the presence of disease within the cavity shavings was a predictor of poorer DDFS, p=0.0010 (relative risk 3.2754, 95% confidence interval 1.6122-6.6544).

Graph 1.3: Linear representation of incidence of Distant Disease-Free Survival (DDFS)



* p=0.0092

Numbers of patients at risk	Time Zero	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year	9th year
Cavity Shaving negative	343	333	274	235	185	125	125	125	125	125
Cavity Shaving positive	143	142	118	102	81	60	60	59	59	59

Table 1.21: Cox regression analysis of Distant Disease-Free Survival (DDFS)

Co-variate	Significance (*p*-value)	Relative risk	95% Confidence interval
Age at diagnosis	0.7631	1.0045	0.9756-1.0342
Maximum tumour diameter	0.3347	1.0189	0.9809-1.0584
Poorer tumour grade	0.9272	1.0145	0.7446-1.3823
Axillary nodal disease	**0.0118**	1.0962	1.0206-1.1775
Oestrogen receptor negativity	**0.0003**	3.2724	1.7333-6.1783
Tumour bed positivity	**0.0010**	3.2754	1.16122-6.6544

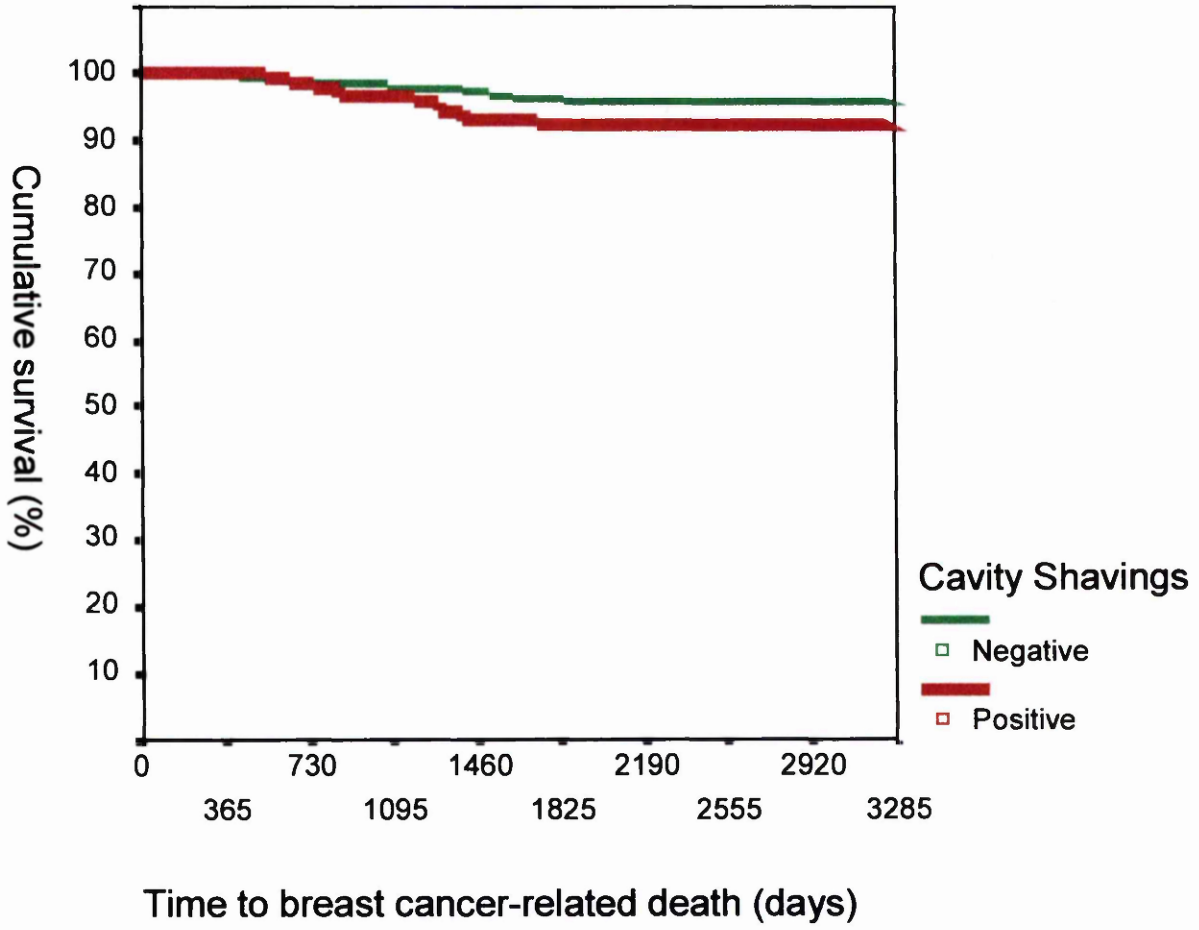
Probability of Overall Survival (OS)

The linear representation of overall survival for the cavity shavings positive and negative cohorts is shown in Graph 1.4. On performing the log-rank test, there was no significant difference between the tumour bed positive and negative patients, p=0.1210.

When cavity shaving status was tabulated against nodal disease, node negative/tumour bed positive patients did worse than tumour bed negative/node negative patients (Graph 1.5). However, this difference was not significant, p=0.2303.

On performing a Cox regression analysis of all the co-variates, only axillary nodal disease predicted for poorer overall survival, p=0.0092 (Table 1.22). This co-variate was then entered into the final model which demonstrated that the presence of cavity shaving disease did not predict for poorer overall survival, p=0.1030.

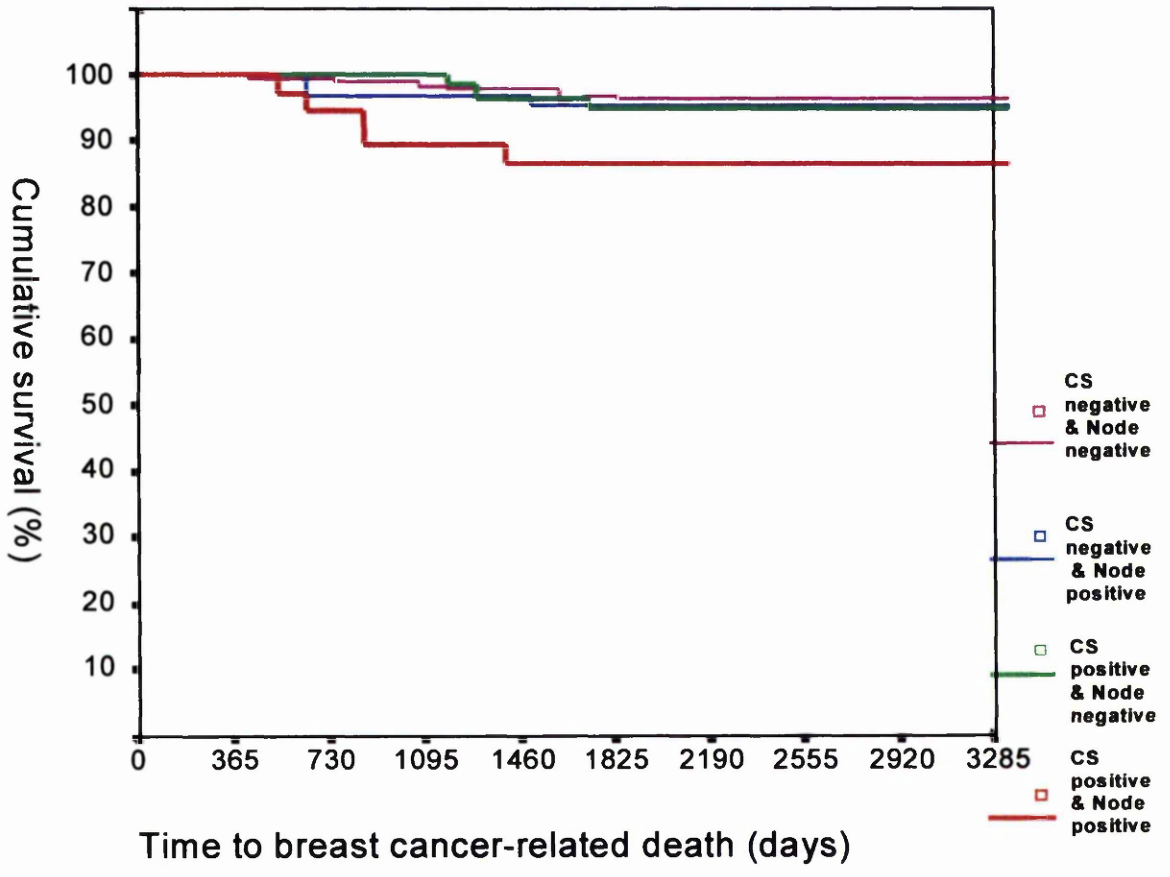
Graph 1.4: Linear representation of incidence of Overall Survival (OS)



* p=0.1210

Numbers of patients at risk	Time Zero	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year	9th year
Cavity Shaving negative	343	343	282	246	199	138	98	65	45	20
Cavity Shaving positive	143	143	122	110	91	69	44	35	23	17

Graph 1.5: Linear representation of incidence of Overall Survival (OS), tabulating cavity shaving (CS) status against nodal disease



* CS = Cavity Shavings

Numbers of patients at risk	Time zero	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year	9th year
CS negative & Node negative	234	234	191	167	133	88	87	87	87	87
CS negative & Node positive	69	69	60	53	45	34	34	34	34	34
CS positive & Node negative	83	83	67	61	52	37	35	35	35	35
CS positive & Node positive	39	39	34	30	23	18	18	18	18	18

Table 1.22: Cox regression analysis of Overall Survival (OS)

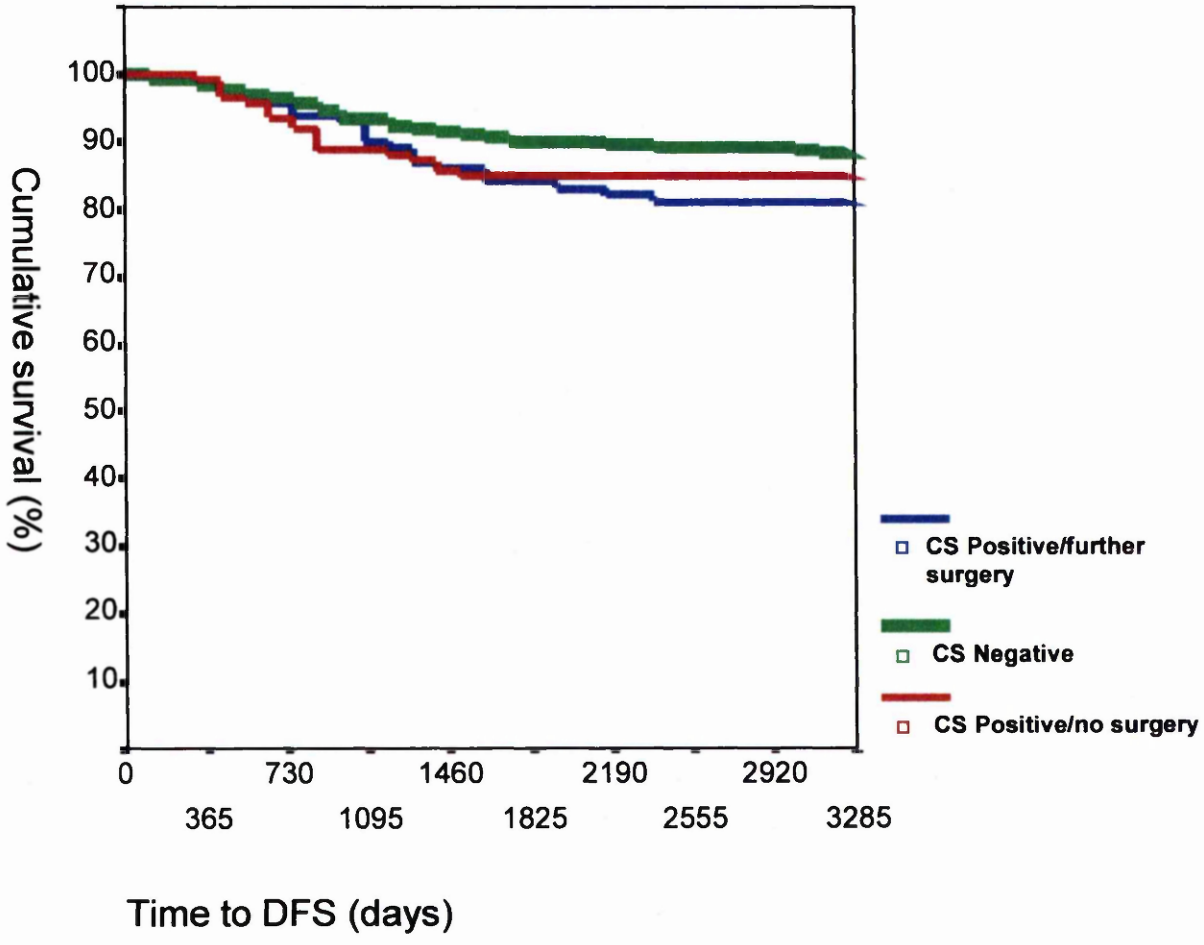
Co-variate	Significance (*p*-value)	Relative risk	95% Confidence interval
Age at diagnosis	0.1754	1.0249	0.9891-1.0620
Maximum tumour diameter	0.1849	1.0363	0.9831-1.0924
Poorer tumour grade	0.2045	1.2858	0.8720-1.8960
Axillary nodal disease	**0.0092**	1.8173	1.1595-2.8481
Oestrogen receptor negativity	0.2794	1.5610	0.6966-3.4979
Tumour bed positivity	0.1030	1.3332	0.9436-1.8836

Effect of extensive tumour bed disease on patient outcome

In all, 57 (10.5%) patients underwent a mastectomy due to extensive residual disease within the tumour bed. Of these patients in 36 (63%) cases this disease was invasive (or mixed invasive/in-situ disease) in nature. Graphs 1.6-1.7 represent the effect on DFS and overall survival of a patient undergoing further surgery, either mastectomy or re-excision.

Of note is the fact that patients undergoing further surgery for residual disease had a poorer DFS and overall survival than those patients who were tumour bed positive but did not undergo further surgery. When the log-rank test was performed however, this difference did not reach significance, p=0.5207 and 0.4605 respectively.

Graph 1.6: Effect of further surgery on Disease-Free Survival (DFS)

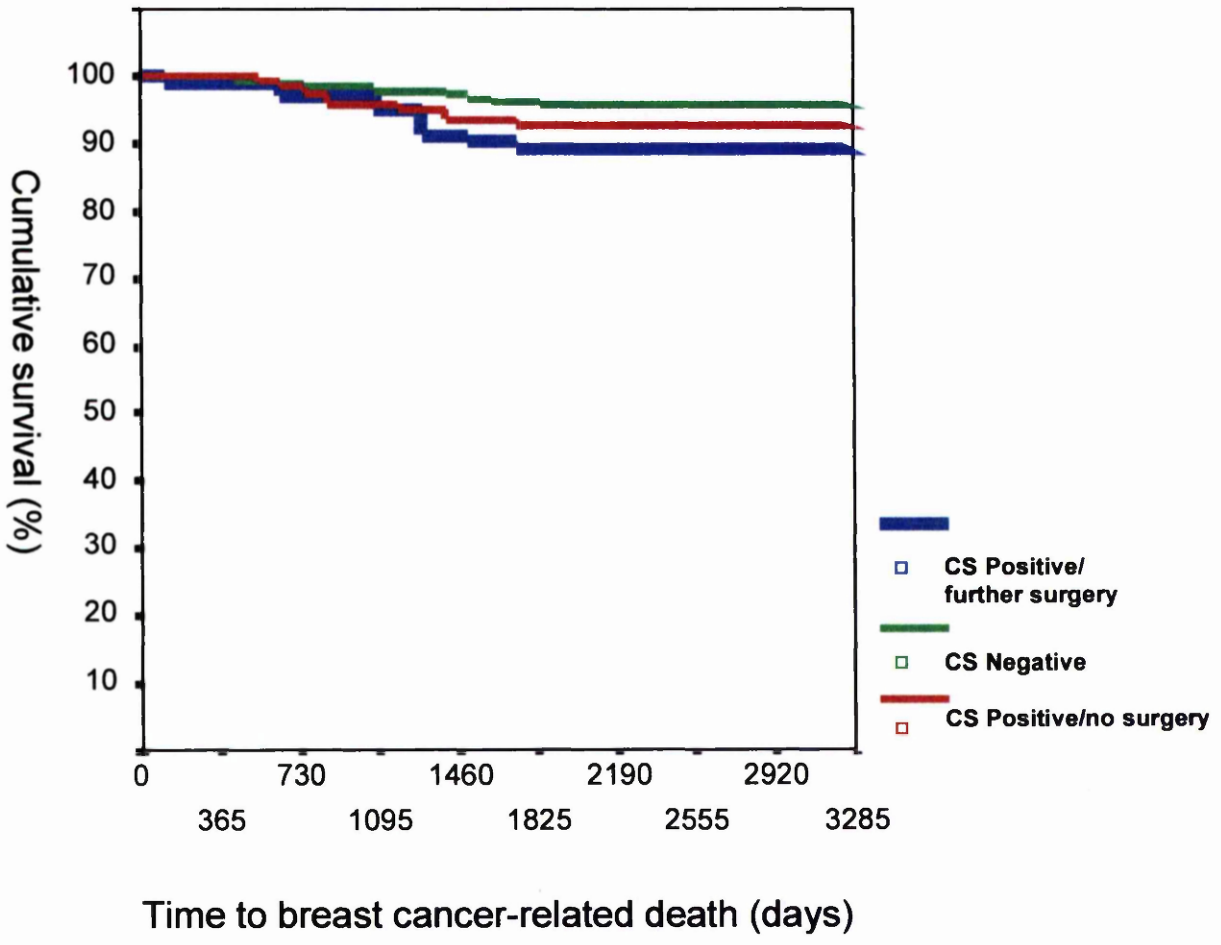


* p=0.5207

* CS = Cavity Shavings

Numbers of patients at risk	Time Zero	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year	9th year
CS Positive/ further surgery	83	82	63	50	33	13	4	3	3	3
CS Negative	343	338	274	232	181	119	79	45	24	2
CS Positive/no surgery	117	117	89	78	56	34	11	2	2	0

Graph 1.7: Effect of further surgery on Overall Survival (OS)



* p=0.4605

* CS = Cavity Shavings

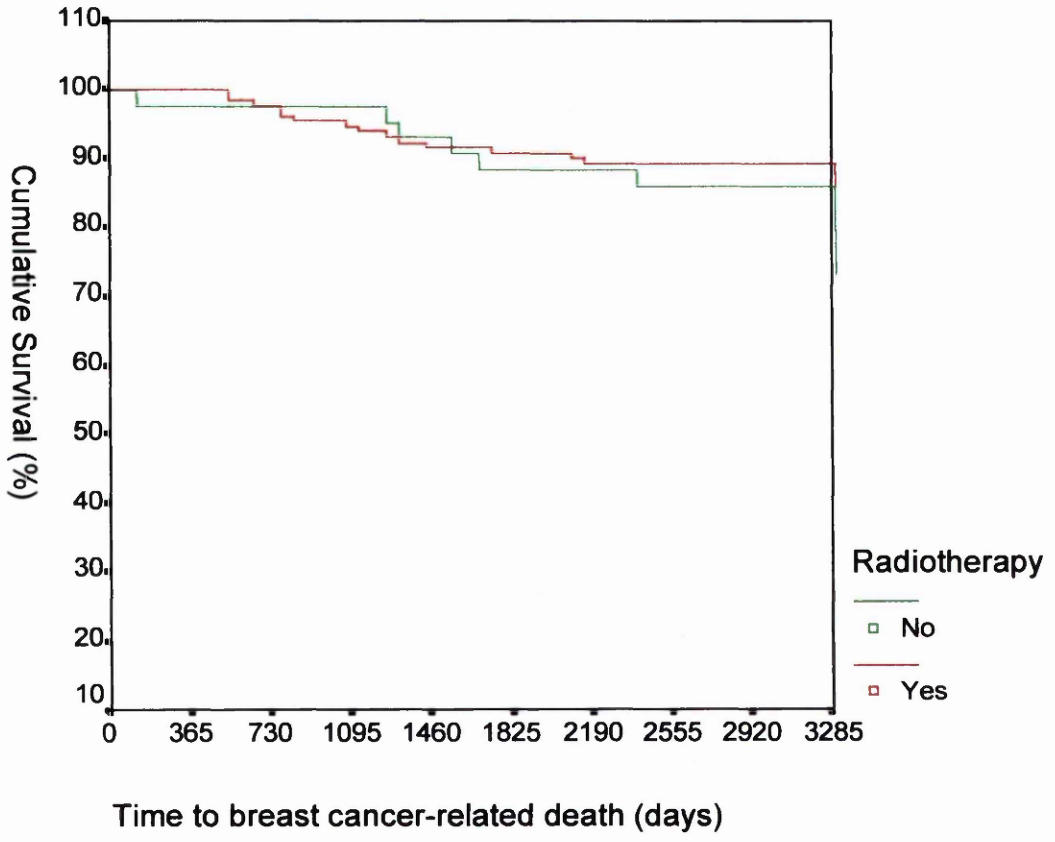
Numbers of patients at risk	Time Zero	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year	9th year
CS Positive/ further surgery	83	82	63	54	38	16	5	4	3	3
CS Negative	343	343	282	246	199	138	98	65	45	20
CS Positive/no surgery	117	117	95	84	65	40	15	4	2	0

Effect of adjuvant therapy on outcome

Of the 543 patients who had tumour bed assessment performed 441 (81.2%) had post-operative radiotherapy. Of the 102 (18.2%) of patients who did not receive radiotherapy, 57 (10.5%) underwent a mastectomy and a further 45 (7.7%) were entered into clinical trials. A further 431 (79.4%) of patients were offered tamoxifen and 61 (11.2%) were offered adjuvant chemotherapy.

On performing the log-rank test, patients receiving post-operative radiotherapy were found to have a slightly better overall survival, p=0.0193 (Graph 1.8). However, receiving post-operative radiotherapy was not found to be beneficial on either DFS or DDFS, p=0.6055 and 0.7652 respectively. There was no association between adjuvant chemotherapy and improved DFS, DDFS or overall survival, p=0.4942, 0.9975 and 0.1841 respectively. Furthermore, receiving tamoxifen was not associated with an obvious improvement in either DFS, DDFS or overall survival, p=0.1206, 0.9448 and 0.5207 respectively.

Graph 1.8: Effect of radiotherapy on Overall Survival (OS)



*p=0.0193

Numbers of patients at risk	Time Zero	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year	9th year
No post-operative radiotherapy	102	101	98	98	93	85	85	84	84	84
Post-operative radiotherapy	441	441	417	401	381	356	353	353	353	353

DISCUSSION

Breast-conserving surgery has become an increasing popular alternative to mastectomy over the last decade. Although several trials have shown that breast-conserving surgery is associated with equivalent rates of survival compared with mastectomy, the problem of local recurrence persists. The major risk factor for the development of local recurrence is inadequate tumour excision. In order to facilitate an improved assessment of the tumour margin following lumpectomy, several techniques have been developed. Assessment of the tumour bed has been shown to be more effective at detecting residual disease than the inking of margins (38,61,162). A previous publication from this institution showed the incidence of tumour bed positivity to be 39.3% (61).

In order to update and re-analyse earlier results we proceeded to undertake this retrospective analysis of this institutions experience in breast-conserving surgery over the previous 15 years. This study addresses three main issues firstly, the incidence of residual disease within the tumour bed after breast-conserving surgery; secondly risk factors associated with tumour bed positivity and thirdly the association between tumour bed positivity and patient outcome. Although the Western Infirmary in Glasgow is a tertiary referral centre no significant difference in the socio-ecenomic distribution of this cohort of patients when compared to the Scottish average was noted.

Among the 543 patients that had cavity shavings performed the incidence of tumour bed positivity was 37%. This figure correlates well with the previously published data (61). In the initial study from this institution by Macmillan et al the only clinico-pathological factor that predicted for tumour bed positivity was poorer tumour grade (61). The results from this study however show that tumour bed positivity is associated with symptomatic presentation; poorer tumour grade; lymphovascular invasion; axillary nodal disease and EIC. Furthermore, when a multivariate analysis is performed poorer tumour grade; axillary nodal disease and extensive in-situ disease within the breast are all predictors of tumour bed positivitry. There may be several factors responsible for this difference in results between these two studies. Firstly, the original study contained only 264 patients. Thus the increase in the numbers of patients analysed in the present study may have resulted in detection of associations that previously did not reach significance. Furthermore, Macmillan's data is gathered retrospectively from 1988 to 1992. Thus this data may also have suffered from inter-observer variation in pathological reporting as only latterly was there a specialist pathologist reporting on all breast cases. One weakness in the current study however is the fact that the histological slides were not reviewed. Thus for some of the earlier patients included in this study a specialist pathologist may not have reported the pathological slides raising the possibility of inaccuracy. Despite this misgiving the current study has shown several clinico-pathological features that predict for a positive tumour margin.

Furthermore, all the risk factors for tumour bed positivity have also been previously shown to be independent risk factors for the development of local recurrence (17,31,32,34-38,42). This raises the possibility that the presence of poorer tumour grade; lymphovascular invasion and extensive in-situ disease within the tumour may be markers of a more locally advanced disease process within the breast. Consequently in such cases a traditional wide local excision may leave behind residual disease that can eventually progress on to a local recurrence.

In this study there was an association between residual invasive disease within the cavity shavings and axillary nodal disease. This result may be due to the fact that invasive disease within the cavity shavings was associated with the presence of lymphovascular invasion. The presence of lymphovascular invasion has previously been found to be a predictor of nodal disease and poorer survival (28,30,32,34,36). This result may however be biased by the sampling error introduced, as results on axillary nodal status were not present in all the patients who underwent axillary clearance.

The finding that oestrogen receptor positivity predicted for disease within the tumour bed was unexpected and may be due to a disproportionately high incidence of oestrogen receptor positivity within this group of patients. This

apparent association did not however reach significance when a multivariate analysis is performed.

In this institution a policy of selective further surgery has been employed when dealing with positive tumour margins. The decision whether to perform further surgery has always been a pragmatic one. Several factors determine whether patients proceed to re-excision or mastectomy following initial wide local excision. These factors include patient choice; general fitness for further surgical intervention as well as both the type and extent of residual disease within the tumour bed. A total of 42% of the patients with tumour bed positivity underwent further surgery, either re-excision or mastectomy. Among the remaining 58% of patients not undergoing further surgery, all had between 1 to 3 foci of disease confined to one block of tissue from the cavity shavings. Of the patients undergoing further surgery residual disease was found in 33.7% of cases. Although these results suggest that a proportion of patients underwent unnecessary procedures, it is not always possible to sample a mastectomy specimen extensively, thus the presence of residual disease can be underestimated. Furthermore, the approach used within this institution is one of prevention. Interestingly in the earlier study by Macmillan et al only 35.6% of patients with a positive margin underwent further surgery compared to 42% in this updated series. This suggests that there has been a gradual change with time towards further surgery if a positive tumour margin is present.

In this study an overall local recurrence rate of 2.3% was observed. This is low when compared with the published data (38,62-64). There was no association between tumour bed positivity and local recurrence. The fact that tumour bed positivity is not a predictor of local recurrence is not surprising as those patients who have extensive disease within the tumour bed often undergo further surgery in order to clear this disease. Thus they can subsequently be regarded as being at a low risk of developing local recurrence.

The systemic recurrence rate in this series was only 2.5%, which is low compared to the published literature (17,31-32,38,56,62-64,162). This may however be accounted by the fact that in the literature the incidence of nodal positivity varies from 33% to 43% (31-32,56,162). In this series however only 29% of the patients had nodal disease. Thus this population may well be skewed towards a better outcome. The increased incidence of non-breast cancer-related deaths seen in the tumour bed positive cohort is a quandary. This may well represent differences in cardiovascular risk factors within this cohort of patients that are out with the scope of this study.

Tumour bed positivity was found to predict for poorer DFS and DDFS but not overall survival. The lack of a relationship between tumour bed positivity and overall survival may however be due to the length of follow-up. With a longer follow-up such a relationship may become apparent. It should be noted that due

to the low local recurrence rate observed, the impact of tumour bed positivity on DFS is due mainly to its effect on systemic recurrence. Although no formal analysis of the impact of local recurrence on patient outcome was performed within this study, it is interesting to note that a positive tumour margin that is a predictor of the adequacy of tumour excision and thus local control, can also have an impact on systemic recurrence. This suggests that there may be a relationship between local and systemic disease. Evidence from the literature seems to back this hypothesis. Results from the NSABP-B06 trial have shown that those patients who go on to develop local recurrence have a strong association with subsequent systemic disease, relative risk of 3.41 (38). Similarly results have been found in the OCOG trial, relative risk of 2.11 (62). Interestingly, patients undergoing further surgery for extensive residual disease within the tumour bed have been found to have poorer DFS and overall survival than those tumour bed positive patients who did not merit further surgery. The effect on poorer survival of tumour bed positivity thus seems to be independent of any residual disease within the breast itself. This suggests that those patients who have extensive residual disease at the onset form a subgroup of patients who have aggressive cancers.

When assessing the effect of adjuvant therapy on outcome it was found that the use of tamoxifen was associated with an improvement in DFS. Furthermore, there was no association between DFS and post-operative radiotherapy. These

results highlight the fact that due to the low local recurrence rate the major impact on DFS is from systemic recurrence.

In summary, the use of cavity shavings is a simple and efficient method of assessing the tumour margin. This technique detects a high incidence of occult disease within the tumour bed. Furthermore, the local recurrence rate observed in this series is favourable, when compared to the published literature. However, the results from this study suggests that adoption of a policy of selective further surgery in order to achieve a low local recurrence rate comes at the expense of a high a re-excision or mastectomy rate. This policy can result in excess morbidity. Furthermore, patients with extensive disease within the tumour bed form a subgroup who have been shown to have poorer outcome and in whom more radical surgery may not be justified. In order to clarify the relationship between tumour bed positivity and local recurrence further prospective study randomising patients with tumour bed positivity to further and no further surgery may well be needed. Such a study would confirm any link between residual disease within the tumour bed and local recurrence thus allowing justification of pursuing an aggressive policy of re-excision.

CHAPTER 2

INTRODUCTION

The aim of breast-conserving surgery in the treatment of breast cancer is to excise the tumour completely and achieve an excellent cosmetic result. This must not however compromise the degree of local control allowed by mastectomy. The decision to opt for breast-conserving surgery is based on several factors including tumour size; site and patient preference. The consequences of inappropriately selecting patients for breast-conserving surgery are re-operation (re-excision or mastectomy) in order to obtain disease-free excision margins or at worst, local recurrence. Data obtained from prospective randomised trials has shown that the incidence of local recurrence varies from 0.3 to 15% in patients receiving post-operative radiotherapy and increases up to 43% in those not receiving post-operative radiotherapy (17,38,62-64). The significance of local recurrence following breast-conserving surgery and its relationship to long term outcome is however the subject of debate.

The risk factors for the development of local recurrence have previously been reviewed (22,166). However, inadequate tumour excision has been recognised as a major risk for the development of local recurrence

(22,166). In order to ensure adequacy of tumour clearance, several groups advocate inking of the specimen with India ink (17,38,167). This method of tumour margin assessment is laborious and involves taking multiple sections from the excision specimen in order to assess whether the tumour crosses or lies "close" to the inked margin. Also, only a small proportion of the excision specimen surface area can be assessed by this method. Furthermore, there is no consensus regarding a positive tumour margin using this technique. An alternative approach to assess the completeness of excision is to look for the presence of residual disease within the breast. The technique of tumour bed assessment involves the histopathological analysis of shavings from the residual cavity following wide local excision of the primary tumour. The techniques of margin inking and tumour bed assessment are not however wholly compatible, as tumour bed assessment will detect residual disease as well as isolated foci of disease distant from the tumour. Inking has been shown to underestimate the extent of residual disease as assessed by analysing the tumour bed (60,162).

Taylor et al reviewed 286 patients who had the tumour margin assessed by performing bed biopsy from the walls of the residual cavity (163). Taylor found that in 22% of cases there was evidence of a positive margin (163). Furthermore, tumour bed positivity was found to predict for local

recurrence (163). Assersohn et al however found no association between involved margins and local recurrence (166). In this study a cohort of only 184 patients was analysed and this number may be inadequate to detect any association between positive margins and local recurrence. Furthermore, in this study margins were assessed by inking, a technique that has been shown to underestimate the extent of residual disease (60,162). This fact may also have influenced Aeeersohn's results.

In summary inadequate tumour excision has been shown to be a major risk factor for the development of local recurrence following breast-conserving surgery. Several techniques have been devised to assess the adequacy of tumour excision. However, tumour bed assessment has been shown to detect a higher incidence of residual disease when compared to inking of margins (60,162). Prior to 1988 in this institution there was no formal policy of margin analysis. From 1988 onwards however, all patients undergoing breast-conserving surgery had tumour bed assessment performed. The aim of this study is to assess the impact of adopting tumour bed assessment combined with a pragmatic policy of selective re-excision on the local recurrence rate. In order to achieve this patients treated pre-1988 were compared with patients treated post-1988, with all patients having a fixed follow-up period.

PATIENTS AND METHODS

Patients

A total of 364 patients were treated by breast-conserving surgery between September 1982 and June 1992. All had stage I or II breast cancer, diagnosed by triple assessment. The mean age of patients was 55years (range 26-81, SD. 10.68 years). All 125 patients treated prior to 1988 presented symptomatically. Of the 239 patients treated post-1988, 136 (56.9%) patients presented symptomatically and the remaining 103 (43.1%) were referred through the National Health Service Breast Screening Programme (NHSBSP).

Surgical technique

The surgical technique has already been described in chapter one. In all patients, the surgeon performed a macroscopic resection of the tumour. From 1988 onwards however, following initial wide local excision, tissue forceps were applied to the wall of the residual cavity and a thin shaving of tissue excised (cavity shavings). This was submitted separately for histopathological analysis. All the cavity shavings were serially blocked, sectioned and analysed.

Further surgery

From 1988 onwards, based on tumour bed positivity, a proportion of patients underwent re-excision. The decision to offer further surgical intervention was a pragmatic one and depended on the type and extent of disease.

The incidence of tumour bed positivity was 30.5% (73/239 patients). A total of 12/73 (16.4%) patients underwent re-excision in light of tumour bed positivity. Of the 61/73 tumour bed positive patients who did not undergo further surgery, all had between 1 and 3 foci of disease, which were confined to one block of the cavity shavings. In 59/61 of these patients the disease was in-situ in nature. In the 2 cases where the disease was invasive, it was restricted to 1 focus of disease in one block.

Adjuvant therapy

In all, 289 (79.3%) patients received post-operative radiotherapy to the breast consisting of 46 Gy in 23 fractions over 35 days with a 12 Gy boost to the tumour bed in 4 fractions over 7 days. The majority of patients who did not receive radiotherapy were those entered into clinical trials and randomised not to receive post-operative radiotherapy. A total of 42 (11.5%) patients received adjuvant chemotherapy and 235 (64.5%) patients received tamoxifen (Table 2.1).

Table 2.1: Adjuvant therapy

Adjuvant therapy	Pre-1988 group (%)	Post-1988 group (%)
Radiotherapy	103 **(82.4)**	186 **(77.8)**
Chemotherapy	27 **(21.6)**	15 **(6.3)**
Tamoxifen	52 **(41.6)**	183 **(76.6)**
Total	**125**	**239**

Follow-up

In order to obtain a full five-year follow-up for both cohorts, patients were followed up prospectively at outpatient clinics. All patients were seen regularly at three monthly intervals for the first year and six monthly intervals thereafter. Two-view mammography was performed every two years. During the five-year follow-up period of this study 4 patients were lost to follow-up in the pre-1988 group and 2 patients in the post-1988 group. All patients lost to follow-up were well at the last clinic visit. In order to obtain a meaningful comparison between these two cohorts of patients, all events occurring at a ***fixed*** five-year follow-up period in each individual patient was documented.

Statistical analysis

The Pearson's chi-squared test and the independent sample t-test were used to assess differences in clinico-pathological factors between the pre- and post-1988 cohorts. A 5% significance level was used in this analysis.

The Kaplan-Meier technique was used to produce a curve of the incidence of local recurrence. The log-rank test was performed in order to

determine underlying differences in the incidence of local recurrence between the groups of patients.

Multivariate analyses using the Cox proportional hazard model (165) was used to produce a set of in-depth useful predictions of local recurrence. The model was performed in a stepwise manner with only co-variates reaching a 5% significance level being included in the final model. The same co-variates as in chapter one were included in the model.

RESULTS

In the pre-1988 non-cavity shavings group the mean age at diagnosis was 55.16 years (S.D=12.06 years) compared with 55.11 years (S.D=10.05 years) in the post-1988 cavity shavings group, p=0.93. In all, 296 (81%) patients presented with invasive ductal carcinoma, 20 (5.5%) with lobular carcinoma, 27 (7.5%) with tumours of special type and in the remaining 21 (6%) the type of tumour was not defined. Oestrogen receptor status was determined on 302 patients. Of these, 204 (67.5%) patients were oestrogen receptor positive. A total of 304 (83.5%) patients underwent axillary surgery (level II clearance) and of these 33% had nodal disease.

Results of patient pathology and any underlying significant associations are presented in Table 2.2. There were more patients in the post-1988 group with smaller, node-negative tumours than in the pre-1988 group. This difference is probably due to the influence of the NHSBSP. Also of note was the apparent increased incidence of oestrogen receptor positivity seen in the post-1988 group. This may be spurious in that only 82/125 (65.6%) patient pre 1988 had receptor status determined compared with 204/239 (85.3%) patients in the post-1988 group.

Table 2.2: Comparison of pathological factors between pre- and post-1988 groups

Pathological factors	Pre-1988 group (%)	Post-1988 group (%)
Ductal carcinoma	85 **(68)**	211 **(88.3)**
Lobular carcinoma	8 **(6.4)**	12 **(5)**
Special type	11 **(8.8)**	16 **(6.7)**
Not defined	21 **(16.8)**	0
Mean tumour diameter (mm)	19	13 ***p*=0.0001**
Oestrogen receptor positive	41/82 **(50)**	163/220 **(74)** ***p*=0.0001**
Node positive	39/97* **(40.2)**	60/204 **(29.4)** ***p*=0.007**
Total	**125**	**239**

* Pathological results of nodal status were available in 97 of the 100 patients who had axillary surgery in the pre-1988 group.

Patient outcome

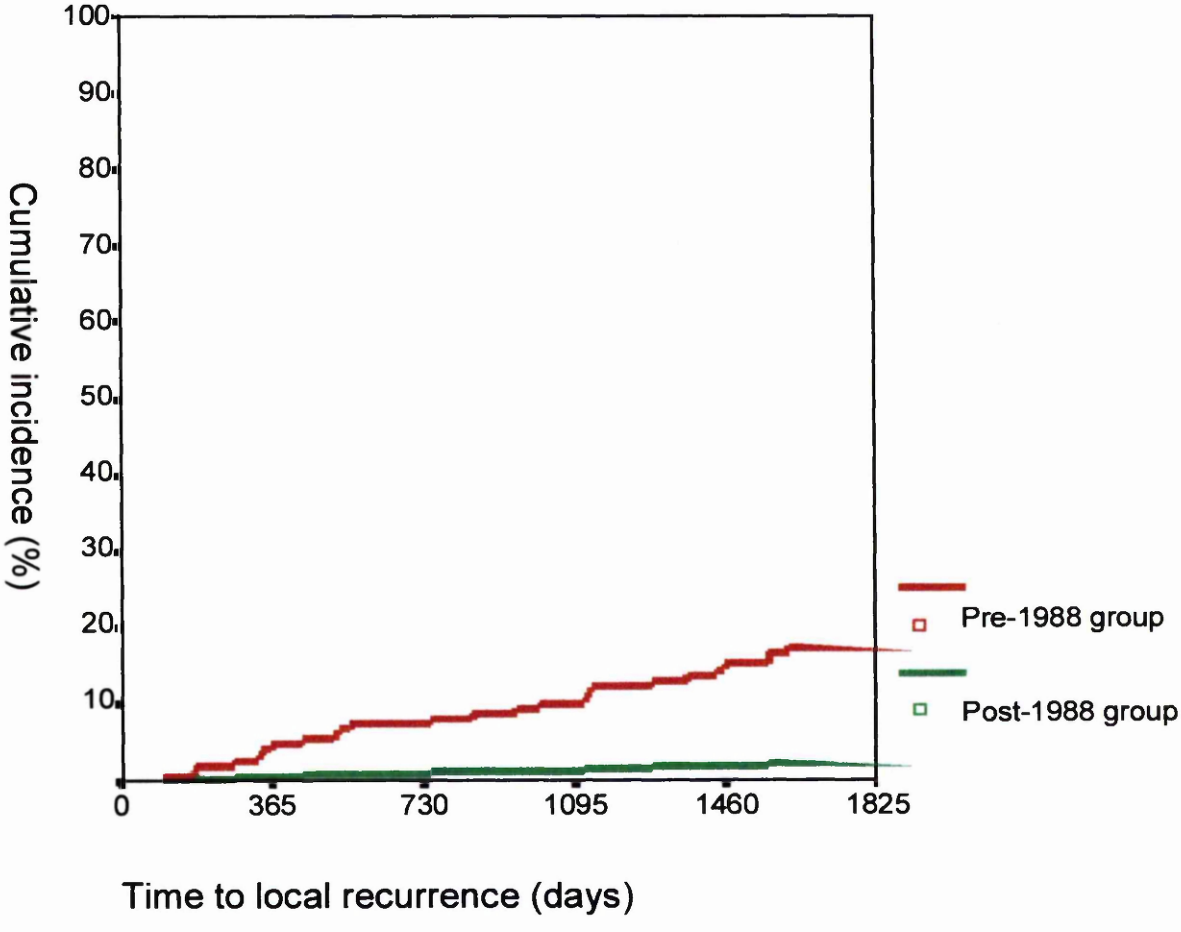
Patient outcome at the end of follow-up is shown in Table 2.3. As can be seen the incidence of local recurrence fell from 15.7% pre-1988 to 2.5% post-1988. The linear representation of local recurrence is shown in Graph 2.1. On performing the log-rank test, the difference in the local recurrence rates between these two groups of patients is significant, $p<0.00001$. Also of note, is that in the pre-1988 group 32% of the local recurrences occurred within 1 year of surgery.

A Cox regression analysis was performed with only oestrogen receptor negativity predicting for local recurrence, $p=0.012$ (Table 2.4). This co-variate was entered into the final model. On performing the final model, the pre 1988 group of patients had significantly poorer local recurrence rates, $p=0.0002$ (relative risk 8.0832, 95% confidence interval 2.7249-23.9777).

Table 2.3: Outcomes after fixed 5 year follow-up in the pre- and post-1988 groups

Outcome	Pre-1988 group (%)	Post-1988 group (%)
Local recurrence	19 (15.7)	6 (2.5)
Contralateral breast tumour	4 (3.3)	1 (0.4)
Axillary recurrence	2 (1.6)	2 (0.8)
Alive with systemic recurrence	9 (7.4)	5 (2.1)
Breast cancer-related death	14 (11.6)	18 (7.6)
Non-breast cancer-related death	3 (2.5)	10 (4.2)
Total	121	237

Graph 2.1: Incidence of local recurrence



* $p<0.00001$

Numbers of patients at risk	Time zero	1st year	2nd year	3rd year	4th year	5th year
Pre-1988 group	121	115	112	108	104	102
Post-1988 group	237	236	235	234	232	231

Table 2.4: Cox regression analysis of local recurrence

Co-variate	Significance (*p*-value)	Relative risk	95% Confidence interval
Age at diagnosis	0.1660	0.9631	0.9131-1.0157
Maximum tumour diameter	0.9830	0.9991	0.9223-1.0824
Poorer tumour grade	0.5764	1.1781	0.6629-2.0937
Axillary nodal disease	0.9167	0.9876	0.7811-1.2485
Oestrogen receptor negativity	**0.0120**	2.7989	1.2539-6.2476
Pre-1988 group	**0.0002**	8.0832	2.7249-23.9777

Effect of adjuvant therapy on local recurrence

In all, 79.3% of patients received post-operative radiotherapy; 11.5% of patients received adjuvant chemotherapy and 64.5% of patients received tamoxifen. The log-rank test was used to assess any relationship between adjuvant therapy and local recurrence.

On performing the log-rank test patients who did not receive adjuvant chemotherapy or tamoxifen had an increased incidence of local recurrence, p=0.003 and 0.0002 respectively. There was however no association found between those who did not receive post-operative radiotherapy and an increased incidence of local recurrence, p=0.4666.

DISCUSSION

Although breast-conserving surgery has been shown to be a safe alternative to mastectomy local recurrence remains a persisting problem. Development of local recurrence has both psychological effects on patient morale as well as effecting patient outcome. Of the risk factors for the development of local recurrence inadequate tumour excision remains paramount. There are several recognised techniques used to determine adequacy of tumour excision. Tumour bed assessment has been shown to detect a high incidence of disease within the tumour bed (60,61,161-163). Furthermore, tumour bed assessment can provide an indication of those patients at risk of developing local recurrence. Preliminary data from this institution has shown that the combination of tumour bed assessment with selective further surgery resulted in a low local recurrence rate (61).

The current study examines the impact of the introduction of a policy of tumour bed assessment in 1988, on local recurrence. In order to achieve this goal data on patients treated pre-1988 was reviewed and compared to the post-1988 results, with all patients having a fixed 5-year follow up. Patients were recruited into this study from 1982 to 1992. In 1987 breast screening was introduced and the combination of this with the fact that these two cohorts were not randomised explains the fact that there were

more patients in the post-1988 group with smaller, node-negative tumours than in the pre-1988 group. Furthermore, there are marked differences in the use of adjuvant therapy, especially chemotherapy and tamoxifen between these two groups of patients. These represent changes in clinical practice as well as differences in clinico-pathological features between these two heterogeneous groups of patients.

The incidence of tumour bed positivity seen in the post-1988 group was 30.5%. This is less that that of 39.3% previously reported by Macmillan et al (61). One explanation for this may be the difference between these two studies in the numbers of patients analysed. Of the patients with a positive margin 16.4% underwent further re-excision for extensive disease within the tumour bed. The remaining patients with a positive margin who did not undergo further surgery all had between 1 to 3 foci disease confined to one block of the cavity shavings. In these patients due to the minimal nature of disease present within the tumour bed a clinical decision was made not to undertake further surgery. Macmillan showed that 35.6% of patients with a positive margin underwent further surgery (61). In the current study the rate of further surgery is almost half of that reported in Macmillan's study. The explanation for this apparent difference is that Macmillan's data included 29 patients that underwent a mastectomy for extensive disease within the tumour bed. However, the

aim of the current study is to analyse local recurrence, thus patients with mastectomy were excluded from the data. However, the rate of re-excision seen in Macmillan's data and the current study are comparable.

The local recurrence rate seen in the post-1988 group is consistent with previously published results (61). A significant fall in the local recurrence rate pre- to post-1988 was observed however. This fall in local recurrence may be due to an improvement in the adequacy of local tumour excision provided by tumour bed assessment. However, these results may be influenced by differences in clinico-pathological factors as well as adjuvant therapy between these two groups of patients. There were significantly more patients in the pre-1988 group that had larger tumour that were node positive and tended to be oestrogen receptor negative. Oestrogen receptor negativity has been shown to be a risk fact for local recurrence (45,46). A similar result has been found in this study. Also, larger tumour diameter has been shown to be a risk for local recurrence in both the Milan and the NSABP-B06 trials (32,16). Furthermore, differences in the extent of local surgical excision practised prior to 1988 may also have influenced the relatively high local recurrence rate witnessed prior to 1988. This is indicated by the fact that 32% of the local recurrences pre-1988 occurred within 1 year of surgery confirming inadequate tumour excision as being a major factor.

There were significant differences in adjuvant therapy between the pre- and post-1988 group of patients. In the pre-1988 group 82.4% of patients received post-operative radiotherapy compared to 77.8% in the post-1988 group. This variation between these two groups is due to a difference in the number of patients being entered into clinical trials and being randomised not to receive radiotherapy. Furthermore, there was no change in the radiotherapy regime over the time frame of this study. Thus differences in the numbers receiving post-operative radiotherapy between these two groups are unlikely to have had an impact on changes in the local recurrence rate that have been witnessed.

Only 41.6% of the pre-1988 group of patients received tamoxifen compared to 76.6% of patients post-1988. This represents a change in clinical practice occurring between pre- and post-1988. This may also have influenced the results. On performing a univariate analysis it was found that patients who did not receive tamoxifen had an increased risk of local recurrence. This result concurs with the literature. The "early breast cancer trialists' collaborative group" published the results from a meta-analysis of the data from 75,000 women with breast cancer (107). This team found that at 5-years follow-up, node negative patients receiving tamoxifen had an annual local recurrence rate of 3.62% compared to 5.22% among those not receiving tamoxifen (107). Similar results were

found for the node positive patients, 11.35% versus 15.29% (107). This benefit to local control was only observed for the first 5 years after starting tamoxifen (107).

Adjuvant chemotherapy was used in 21.6% of patients pre-1988 compared to only 6.3% of patients post-1988. This is almost certainly due to the fact that the pre-1988 group consisted of patients with larger often oestrogen receptor negative and node positive patients. The higher incidence of systemic recurrence and breast cancer-related death seen in the pre-1988 group of patients reflects this. Furthermore, the results from this study show that chemotherapy had a protective effect against local recurrence. This finding concurs with the results from a meta-analysis of 11,000 women with breast cancer randomised to poly-chemotherapy from 31 trials (107). In this study data was also present on a further 8000 women from 16 separate trials randomised to receive single agent versus no chemotherapy (107). The study found that the use of chemotherapy was associated with a protective effect against local recurrence (107).

In summary this study has shown that tumour bed assessment detects a high incidence of occult disease within the breast. Furthermore, the combination of tumour bed assessment with selective further surgery may have resulted in a fall in the local recurrence rate witnessed post-1988.

However, due to the retrospective, non-randomised design of this study the pre- and post-1988 groups of patients are heterogeneous nature. Subsequently the fall in local recurrence is multifactorial in origin. It may be accounted by the impact of tumour bed assessment resulting in an improvement in the adequacy of tumour excision. However differences in clinico-pathological features as well as adjuvant therapy between the pre- and post-1988 groups almost certainly had an influence on local recurrence. In order to analyse this subject further there needs to be performed a prospective trial where patients are randomised to either tumour bed assessment or no formal margin analysis. However, as adequacy of tumour excision has been shown to be a major risk for the development of local recurrence such a study would raise ethical concerns.

CHAPTER 3

INTRODUCTION

Over the past decade breast-conserving surgery has become an increasingly popular alternative to mastectomy in the management of operable breast cancer. However, local recurrence remains a persistent problem. Clinico-pathological factors recognised as predictors for the development of local recurrence include inadequate tumour excision; extensive in-situ disease within the tumour (EIC); poorer tumour grade and the presence of lymphovascular invasion. It is vital to select only those patients appropriate for breast-conserving surgery, as inappropriate patient selection is associated with the morbidity entailed with further surgical procedures in order to ensure adequacy of tumour clearance. The factors commonly used in determining a patient's suitability for breast-conserving surgery are patient choice as well as the clinical size and location of the tumour within the breast. Mammography is an essential tool in the pre-operative assessment of patients undergoing surgery for breast cancer. In addition to revealing diagnostic radiological abnormalities, pre-operative mammographic features may also determine those patients that should be suitable for breast-conserving surgery. Several studies have found a correlation between pre-operative

mammographic features and clinico-pathological risk factors for the development of local recurrence (169-177). These results have the potential in aiding the clinician in selecting those patients that are suitable for breast-conserving surgery.

Healey et al analysed the results of pre-operative mammograms from 101 patients undergoing breast-conserving surgery (169). Within this group, 41 (40.5%) patients showed evidence of EIC (169). The presence of EIC has been shown to be an independent risk factor for the development of local recurrence (34,37). Healey et al found that EIC was associated with the presence of microcalcification as well as an absence of a mammographic nidus (169). Furthermore, when Stomper et al reviewed the results of 101 pre-operative mammograms, the presence of mammographic microcalcification measuring greater than 3cm in diameter was also associated with EIC (170).

There have also been several studies published looking at the relationship between mammographic features and the incidence of positive margins following breast-conserving surgery (171-175). Unfortunately some of these studies contain small numbers of patients making interpretation of the results limited (171-172). Morrow et al however published the results of 263 patients with stage I and II breast cancer that were suitable for

breast-conserving surgery (173). Morrow found that of this cohort, 97.3% of patients were able to remain conserved (173). However, of the patients with an absent mammographic nidus this was only possible in 38% of cases due to the presence of positive margins (173). Similarly, Kollias et al found that histologically involved margins were associated with both the presence of microcalcifications and the absence of a mammographic nidus (174). Kini et al reviewed the data on 400 patients undergoing breast-conserving surgery and found a local recurrence rate within this population of 9.2% (175). When Kini et al reviewed the results from 214 mammograms available for analysis, the incidence of positive margins among patients with mammographic microcalcification was 22% compared to 6% for those without (175).

A previous study from this institution examined the correlation between pre-operative mammographic features and risk factors for the development of local recurrence (29). Macmillan et al reviewed the pre-operative mammographic features from 231 patients undergoing breast-conserving surgery (29). All mammograms were independently reviewed by a single pathologist blinded to the clinico-pathological features of these patients (29). In this series the incidence of EIC was 22.1% (29). The presence of casting calcification, absent mammographic nidus as well as a stellate nidus all predicted for EIC (29). Furthermore, the

incidence of positive tumour margins within this study was 40% (29). This correlates well with the previously published literature from this institution (61). The presence of a DY mammographic pattern (178); casting microcalcification and the absence of a mammographic nidus were all found to be risk factors for the presence of residual disease within the tumour bed (29). When all three of these mammographic features were present however, the incidence of tumour bed positivity rose from 40% to 89% (29).

In summary, certain mammographic features have been shown to detect clinico-pathological risk factors for the development of local recurrence. Thus mammography can provide the clinician with a valuable tool that aids in the selection of those patients suitable for breast-conserving surgery. The aim of this study is to expand upon previous work linking pre-operative mammographic findings with established clinico-pathological risk factors for the development of local recurrence.

PATIENTS AND METHODS

Patients

Of the 543 patients who had tumour bed assessment performed (chapter one) pre-operative mammographic data was available on 357 patients (Table 3.1). The mean age of patient was 56 years (range 27-83, S.D 9.37). All patients had breast-conserving surgery performed and of these, 307 (86%) patients had a level II axillary clearance performed.

Table 3.1: Clinico-pathological features

Clinico-pathological features	
Symptomatic presentation	237 **(66)**
Screen-detected palpable tumours	22 **(6)**
Screen-detected impalpable tumours	98 **(28)**
Invasive ductal carcinoma	301 **(84)**
Lobular carcinoma	22 **(6)**
Special type	34 **(10)**
Axillary nodal disease	77/307 **(25)**
Oestrogen receptor positivity	250 **(70)**
Total number of cases	**357**

- Values in parentheses are percentages

Surgery and pathological assessment

Surgical technique and pathological assessment has been described in chapter one.

Mammographic interpretation

All patients in this study had bilateral cranio-caudal (cc) and oblique mammograms performed pre-operatively. All mammograms were reviewed by a single radiologist blinded to the pathological results. Table 3.2 represents the mammographic features assessed in all patients. Illustrations 3.1-3.4 represent some of the pre-operative mammographic features identified in this group of patients.

Statistical analysis

The relationship between clinico-pathological factors and tumour bed positivity with mammographic features was analysed using the Pearson's chi squared and the Independent sample t-tests. In order to perform a multivariate analysis of the mammographic factors that predict for tumour bed positivity a stepwise logistic regression analysis was performed.

Table 3.2: Mammographic features assessed in all patients

Mammographic features	
Side of lesion	Right Vs Left
Number of lesions	
Breast quadrant	UOQ, UIQ, LOQ, LIQ and Retroaerolar
Clock	
Breast density*	Fatty (N1) : Mixed (P1&P2): Dense (DY)
Lesion type	Spiculated mass : dense nidus Spiculated mass : fatty nidus Ill-defined non-spiculated mass Partly-defined non-spiculated mass Well-defined non-spiculated mass Distortion Asymmetry Microcalcification
Micro-calcification	Present vs absent
Micro-calcification type	Casting vs non-casting
Size of nidus (excluding spicules)	Oblique view mm CC view mm Maximum diameter mm
Total size of lesion (including spicules)	Oblique view mm CC view mm Maximum diameter mm
Nidus to nipple distance	Oblique view mm CC view mm Maximum diameter mm
Breast size	Oblique view mm CC view mm

* For the purposes of this study P1 and P2 type Wolfe patterns were grouped together to form the "mixed" mammographic pattern.

Illustration 3.1: Dense mammographic pattern with absent nidus and presence of casting calcification

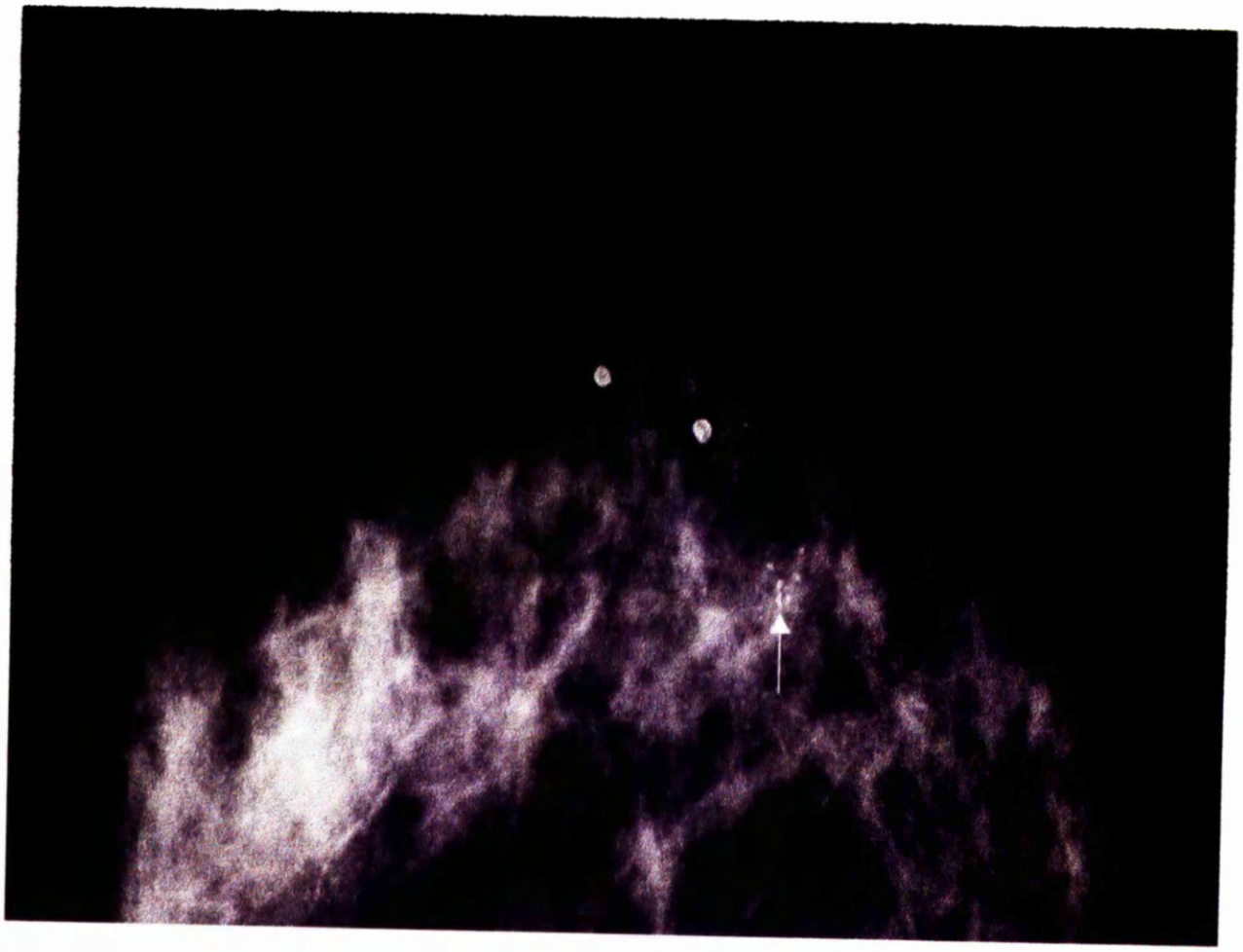


Illustration 3.2: Fatty mammographic pattern with non-stellate lesion

Illustration 3.3: Casting microcalcification

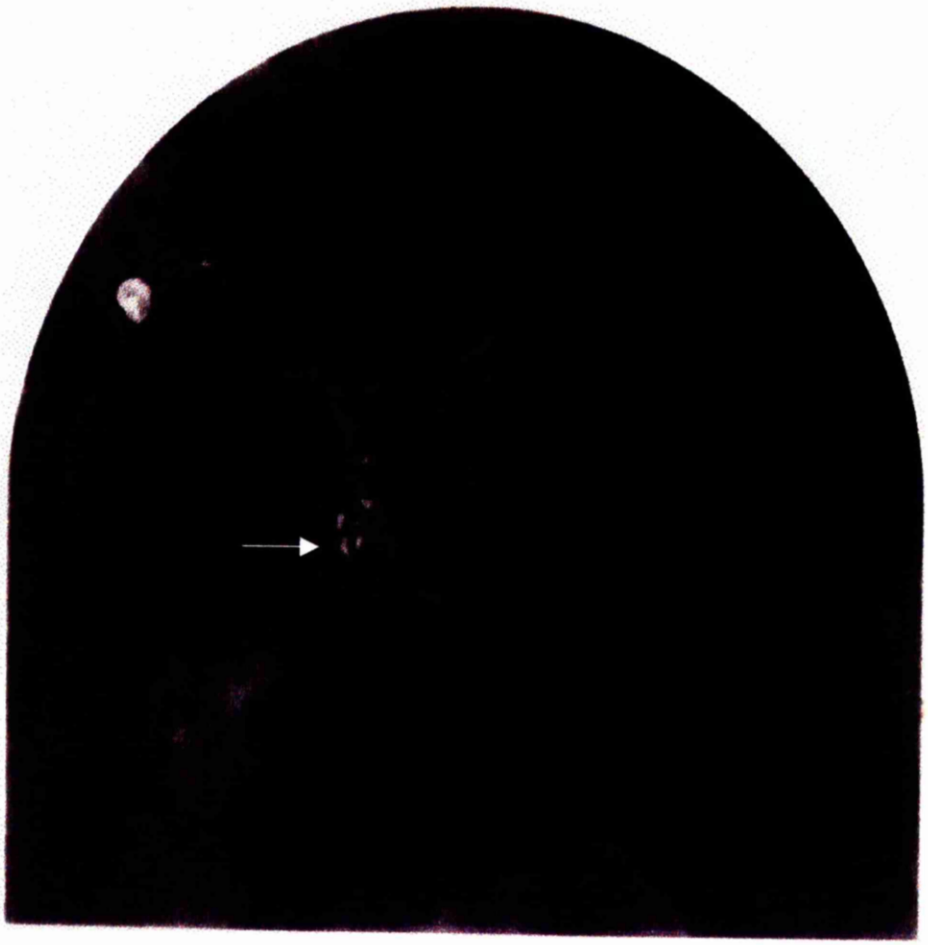
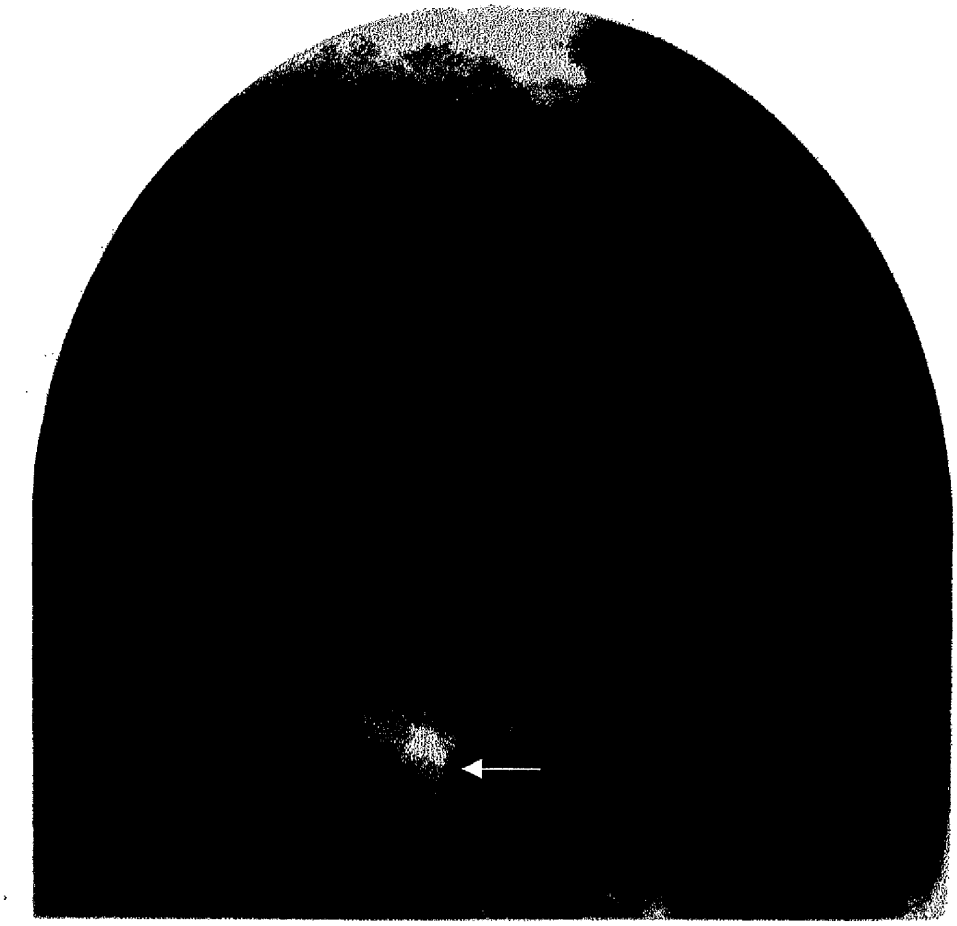


Illustration 3.4: Stellate lesion



RESULTS

Correlation between mammographic features and clinico-pathological factors

The mean age of patient with a dense mammogram was 52 years compared with 56 years for a fatty mammographic pattern, p=0.027. No association was found between type of mammographic abnormality – for example the combination of an absent mammographic nidus with the presence of casting calcification and mode of presentation.

The presence of extensive in-situ disease within the tumour was associated with both casting calcification and absence of a mammographic nidus (p=0.0001 and 0.002 respectively) but not with mammographic density. Furthermore, no association was found between absent mammographic nidus, casting calcification or breast density with tumour grade or type.

Correlation between mammographic features and tumour bed positivity

All patients in this study had tumour bed assessment performed and the incidence of tumour bed positivity was 37%. The correlation between tumour bed positivity and pre-operative mammographic features is presented in Table 3.3. Of note was the association between casting calcification (p=0.003), absence of a mammographic nidus (p=0.027), maximum nidus size (p=0.014) as well as the ratio of maximum mammographic abnormality to breast size (p=0.017 cranio caudal view and p=0.045 oblique view) with tumour bed positivity. No association was found between breast density and tumour bed positivity.

The overall incidence of tumour bed positivity in this group of patients was 37. However, when both an absent mammographic nidus and casting calicification were present the incidence of tumour bed positivity rose to 60%.

Table 3.3: Correlation between mammographic features and tumour bed positivity

Mammographic feature	Tumour bed positive	Tumour bed negative
Breast density		
Fatty (N1)	18 **(31)**	39 **(69)**
Mixed (P1 & P2)	78 **(37)**	133 **(63)**
Dense (DY)	36 **(40)**	53 **(60)**
Calcification		
Casting	35 **(56)***	28 **(44)** * p=**0.003**
Non-casting	23 **(34)**	45 **(66)**
Absent	74 **(33)**	152 **(67)**
Nidus		
Stellate	62 **(40)**	93 **(60)**
Non-stellate	51 **(31)**	115 **(69)**
Absent	19 **(53)***	17 **(47)** *p=**0.027**
Nipple to nidus oblique (mm)	80	76
Nipple to nidus cc (mm)	79	75
Maximum mammographic abnormality (mm)	35	32
Maximum nidus size (mm)	19*	16 p=**0.014**
Breast size oblique (mm)	100	107
Breast size cc (mm)	91	96
Ratio maximum mammographic abnormality to breast size (oblique)	0.27*	0.22 p=**0.045**
Ratio maximum mammographic abnormality to breast size (cc)	0.32*	0.25 p=**0.017**
Total number of cases	**132**	**225**

* Indicates significant values

- Values in parentheses are percentages

Multivariate analysis of predictors of tumour bed positivity

A multivariate analysis of all the factors which predicted for tumour bed positivity was performed, Table 3.4. On performing this analysis only the presence of casting calcification predicted for tumour bed positivity, p=0.0275 (relative risk 2.7169, 95% confidence interval 1.1917-6.1941).

Table 3.4: Multivariate analysis of predictors of tumour bed positivity

Factor	Significance (*p*-value)	Relative Risk	95% Confidence Interval
Maximum nidus size (mm)	0.0582	0.9629	0.9259-1.0013
Absent nidus	0.4060	1.0309	0.3750-2.8337
Casting calcification	**0.0175**	2.7169	1.1917-6.1941
Ratio nidus/breast size (oblique view)	0.7854	0.01	0.0033-2.9987
Ratio nidus/breast size (cc view)	0.2426	0.5	0.1563-1.5991

DISCUSSION

Local recurrence is a matter of concern to both the surgeon and patient. It not only undermines patient morale it often leads to a mastectomy, thus negating one of the main goals of breast-conserving surgery. Over recent years however, local recurrence is being seen in a more sinister light as the harbinger of systemic disease, even if a causal relationship between these two factors has yet to be determined. Of the risk factors for local recurrence inadequate tumour excision remains paramount. Tumour bed assessment has been previously shown to be able to detect a high incidence of residual disease within the tumour bed (61).

Due to the importance of avoiding local recurrence it is vital to scrutinise patients prior to offering breast-conserving surgery. The usual parameters that determine the choice of surgery apart from patient preference are tumour size and position within the breast. However, there have been studies showing certain pre-operative mammographic features that predict for clinico-pathological risk factors for the development of local recurrence. The presence of microcalcification, as well as absence of mammographic nidus, have been found to be predictors for the presence of EIC (169-170). These mammographic features have also been found to be risk factors for positive tumour margins (171-175). This institution

previously reported results showing that presence of casting calcification, absent mammographic nidus and a dense mammographic pattern were all risk factors for the presence of positive tumour margins (29). Similarly EIC was associated with both casting calcification and absence of a mammographic nidus (29).

This study confirms the earlier findings with regards to predictors of a positive tumour margin and EIC. An association was also found between the size of the mammographic abnormality and positive tumour margin. On performing a multivariate analysis however only the presence of casting calcification predicted for positive tumour margins. Unlike the previous study the presence of a dense mammographic pattern was not found to be associated with tumour bed positivity. This finding may be due to inter-observer variation as a different radiologist interpreted mammographic findings in the current when compared to the previous study. Furthermore, the larger numbers of patients analysed in the current study may also have influenced this result.

The findings from this work have important implications for the pre-operative identification of those patients unsuitable for breast-conserving surgery. Recent developments in radiological imaging techniques such as magnetic resonance imaging have already shown their ability to detect

extensive disease throughout the breast that makes such patients unsuitable for limited resections (179-188). Buuchberger et al prospectively performed magnetic resonance imaging on 254 patients and found that 30% of patients had extensive disease throughout the breast making such patients unsuitable for breast-conserving surgery (179). Furthermore, Palinedo et al compared mammography with magnetic resonance imaging and found that the sensitivity of detecting occult disease with magnetic resonance imaging was greater than that with conventional mammography 91% versus 60% (180). However, this result was only observed among patients with palpable lesions (180). One fact that may explain this difference is that this study contained only 56 patients (180). A larger trial may be needed for the sensitivity of magnetic resonance imaging in impalpable lesions to reach significance. Furthermore, Date et al found that magnetic resonance imaging was able to detect intra-ductal extensions up to 1cm from the primary tumour in 52% of cases (188).

In summary this study has demonstrated several pre-operative mammographic features that predict for both EIC and positive tumour margins. These findings can be used to assist the clinician in identifying those patients who may be unsuitable for breast-conserving surgery. Furthermore a positive tumour margin is regarded as a risk factor for the

development of local recurrence, the onset of which has been shown to be a predictor of poorer survival. Thus the presence of pre-operative mammographic features predictive of tumour bed positivity may also be a surrogate marker for a biologically more aggressive tumour. This finding has obvious implications for subsequent adjuvant therapy offered to such patients. Despite this, the importance of accurate assessment of the tumour margin as well as rigorous follow up cannot be underestimated.

CHAPTER 4

INTRODUCTION

The effect of local recurrence on patient survival is a matter of intense debate. Recent studies have shown that there may be a link between the development of local and systemic recurrence (32,38). However it is difficult to determine any such correlation. Risk factors such as tumour size; lymphovascular invasion and tumour grade and type that predict for local recurrence, are also risk factors for the development of systemic recurrence. This aligned to the fact that when considering the effect of local on systemic recurrence the impact of lead-time bias has to be taken into account makes any study setting out to determine such a correlation extremely difficult to design.

Data collected from the NSABP-B06 trial has shown marked differences in the rates of local recurrence rates between those offered post-operative radiotherapy and those who were not (38). However, despite these differences in local recurrence rates, there was no significant difference in overall survival among the various treatment arms of this trial (38). However, when patients who suffer a local recurrence were analysed as a separate population and outcomes assessed, it was noted that the relative

risk of developing systemic disease increased to between 1.2 to 6.6 (32,38). In the NSABP-B06 trial when a time dependent multivariate analysis was performed the presence of local recurrence was found to be a predictor of systemic recurrence (38). Veronesi et al found similar results when they reviewed their experience of 2233 patients (32). In the NSABP-B06 trial it was shown that patients with local recurrence occurring within 1 year of the initial surgery had a higher incidence of systemic recurrence (38). Similarly, Veronesi showed the relative risk of developing systemic disease to be 6.6 if the local recurrence had occurred within one year of surgery (32). This risk fell to 2.2 and then to 1.2 for local recurrences occurring firstly 2 and then 3 years following surgery (32). Furthermore, analysis of results of the OCOG trial has shown that local recurrence is associated with poorer survival (62). Similarly, Chauvet et al showed that the 5-year survival was 87.5% for those with local recurrence compared to 91.3% for those without (115). These results have been repeated in other studies (31,116-126).

Recognised risk factors for local recurrence include poorer tumour grade; extensive in-situ disease within the tumour and lymphovascular invasion. However the major risk factor for developing local recurrence is inadequate tumour excision. The technique of tumour bed assessment has been shown to detect a high incidence of residual disease following wide-

local excision (61). Macmillan et al showed that tumour bed positivity was a predictor of poorer distant disease-free survival (189). This group also found that patients who underwent further surgical excision for a positive tumour margin had the poorest distant disease-free survival (189). This fact suggests that patients with a positive margin may form a subgroup with aggressive tumours and that the poor distant disease-free survival seen in this group may be independent of residual disease within the breast. This effect on poorer survival appears to be independent of axillary nodal status (189).

Over the past decade there has been an increased interest in the detection of occult axillary nodal disease as detected by immunohistochemistry (IHC) and polymerase chain reaction techniques (PCR). Cote et al analysed the lymph nodes from 736 patients with node-negative breast cancer (190). The nodes were serially sectioned and stained by both Haematoxylin and Eosin as well as by IHC (190). This team found that IHC detected occult disease in 20% of cases compared to 7% with Haematoxylin and Eosin staining (190). Other teams have found similar results (191-192). Although IHC has been found to detect an increased incident of occult disease, the results from the literature seem to be varied. In a review by Dowlatshahi et al IHC appeared to increase the detection rate of occult disease by between 9-33% (193). This variation

may in part be explained by the differing numbers of patients included in these diverse studies as well as differences in the types of antibodies used. Similar variations in the detection rate of occult disease by PCR have been noted 9 to 66% (194-197). Unfortunately due to the use of differing markers for PCR as well as the relatively small numbers included within the above studies a consensus regarding the effectiveness of PCR is difficult to establish. However, when Noguchi et al performed a study directly comparing the two techniques, PCR was able to detect occult disease that was missed on IHC (198).

The impact of occult axillary disease on patient outcome has been a matter of some debate. This subject has been made more difficult by the fact that in the literature occult tumour deposits have been categorised as being either micro- or macro-metastases with varying cut of points of between 0.2 to 2mm (193). Furthermore, some of these studies contain relatively small cohorts of patients making a statistical analysis of survival difficult to interpret (192, 196,199). Despite these reservations several studies have found an association between occult axillary disease and poorer survival (190,192,196,199-201). Cote et al found that the presence of occult disease detected by IHC was a predictor of poorer distant-disease free survival, relative risk of 1.79 (190). This relationship however was only significant among post-menopausal women (190). A

study by the "international breast cancer study group" reviewed the nodal pathology of 921 patients and found that occult disease was present in 9% of cases (201). This group found that occult disease was a predictor of poorer overall survival (201). Elson et al however found no such relationship (191). This study was however based on a relatively small cohort of 97 patients thus making any statistical analysis of outcome difficult to interpret (191).

The type of occult disease found within the axilla may also have a bearing on patient outcome. Hartveit et al reviewed the nodal pathology from 1069 patients and found that 15% had occult disease as detected by serial sectioning of the node (200). When these patients were further analysed occult disease within the nodes was found to be one of two variants, either deposits within the sub-capsular tissue or within the lymphoid tissue itself (200). Hartveit et al found that patients with micro-metastases within the lymphoid tissue had a better outcome compared to those with disease confined to the capsule (200). This study is however hampered by that fact that neither IHC nor PCR was used in the analysis of nodal tissue. Thus Hartveit's team may well have underestimated the incidence of occult disease thus biasing their results. Lockett et al found that the extent of marker positivity on PCR predicted for poorer survival (196).

However this study dealt with a small cohort of only 37 patients in whom any statistical comment on outcome would be inaccurate (196).

In summary local recurrence has been shown to be a predictor of poorer survival. Inadequate tumour excision as detected by tumour bed assessment is one of the major risk factors for the development of local recurrence. Furthermore, Macmillan et al showed that tumour bed positivity was a predictor of poorer survival and that this relationship was independent of the axillary nodal status (189). The aim of this study is to assess whether there is a correlation between tumour bed positivity and occult disease within the axilla, a feature that would help explain the poorer outcome observed in patients with a positive margin.

PATIENTS AND METHODS

Patients

The data presented in this study relates to the cohort of patients dealt with in chapter one. A decision was made to initiate a pilot study designed to assess the relationship between tumour bed positivity and occult nodal disease. A subgroup of the 543 patients from chapter one was chosen for this study. This involved a "study" as well as a "control" group. The numbers included in the "study" group were all those patients who had cavity shavings performed, were node negative and who went onto develop systemic recurrence. This cohort consisted of 19 patients. Of these, 10 patients had positive cavity shavings.

The control group consisted of 19 patients again all of whom had no axillary nodal disease and were alive and well at the end of follow-up. These patients were matched with the study group according to cavity shaving status; tumour type and grade; pathological tumour diameter; oestrogen receptor status and age at diagnosis.

Follow-up

The follow-up of patients in this study range from 15 to 100 months (mean 43 months). The mean follow-up of the study group was 33 months compared to 48 months for the control group.

Statistical analysis

The Pearson's chi-squared and Independent sample t-tests were used to assess any significant differences between the study and control group's clinico-pathological factors. These tests were also used to correlate clinico-pathological features and tumour bed positivity with the presence of occult axillary nodal disease. A 5% significance level was used throughout this analysis.

Immunohistochemistry

In order to enhance detection of occult disease a decision was made in conjunction with a specialised breast pathologist to re-section lymph node specimens at a 3-micron interval compared to the conventional 5-micron interval currently being used within this institution. Furthermore, multiple sections of each block of tissue were prepared for conventional histological examination. Due to the availability of resources within the pathology department after discussion with the specialist pathologist involved with this study a decision was made to use IHC rather than PCR to detect occult disease.

A total of 276 blocks of tissue containing 392 nodes were examined. A total of four slides were prepared from each block of tissue with two being used for immunohistochemical staining and one being used for Haematoxylin and Eosin preparation. Two antibodies that are routinely used within this institution in the analysis of breast cancer were used to stain the tissue, MNF 116 a cytokeratin marker (provided by Daco: 1:125 dilution) and MUC 1 CORE that stains for mucin (provided by Novocastra: 1:125 dilution). The fourth slide from each block was left unstained but underwent the process of fixation as a negative control to ensure that the technique of preparation of the slides did not influence the

outcome. Positive controls of breast cancer tissue were also included in order to assess the efficacy of the antibodies used. An independent pathologist who was blinded to overall patient histology and outcome assessed the prepared slides.

Following sectioning the slides were ovened for at least 30 minutes at 60 degrees centigrade. The slides were then "dewaxed" by being placed in Xylene for 10 minutes and then rinsed in alcohol (3 changes) and finally rinsed in tap water for a few seconds. Endogenous peroxidase activity was then quenched by placing the slides in a 3% aqueous solution of hydrogen peroxide for 10 minutes.

In the case of the MUC 1 antibody antigen retrieval was achieved by microwaving the tissue for a period of 10 minutes. During this process the slides were placed in plastic containers filled with 10mM citrate buffer (pH 6). In the case of the MNF cytokeratin antibody however, antigen retrieval was achieved by placing the slides in a water bath at 37 degrees centigrade in a solution of 0.1% trypsin and 0.1% calcium chloride. The slides were then left to incubate for 10 minutes following which they were rinsed in cold water. The next step involved application of the "blocker", namely normal horse serum (1:60 dilution). After incubation with the "blocker" for 10 minutes the primary antibody was

applied. The slides were then left to incubate for 30-45 minutes at room temperature. Following this step the secondary antibody, namely biotinylated rabbit anti-mouse (1:400 dilution in normal horse serum) was added and the slide left to incubate at room temperature for a further 30 minutes. The sections were then washed for 30 minutes before the third layer was applied. Streptococcal ABC antibody (1:100 dilution) was then added and the slides were incubated for a further 30 minutes at room temperature. The slide was then washed for a further 30 minutes. Visualisation of the antibody was performed by the addition of diaminobenzadine (DAB) to the slide which was then left to incubate for a further 10 minutes in a "safety cabinet" before being finally rinsed in water prior to mounting.

Illustration 4.1 represents a diagrammatic representation of the antibody/antigen binding process. Illustration 4.2-4.3 represents MNF 116-cytokeratin and MUC-1 CORE positive controls. Illustrations 4.4-4.6 represent tissue containing occult disease stained by H&E, MNF and MUC-1 representatively.

Illustration 4.1: Diagrammatic representation of the antibody to antigen binding process

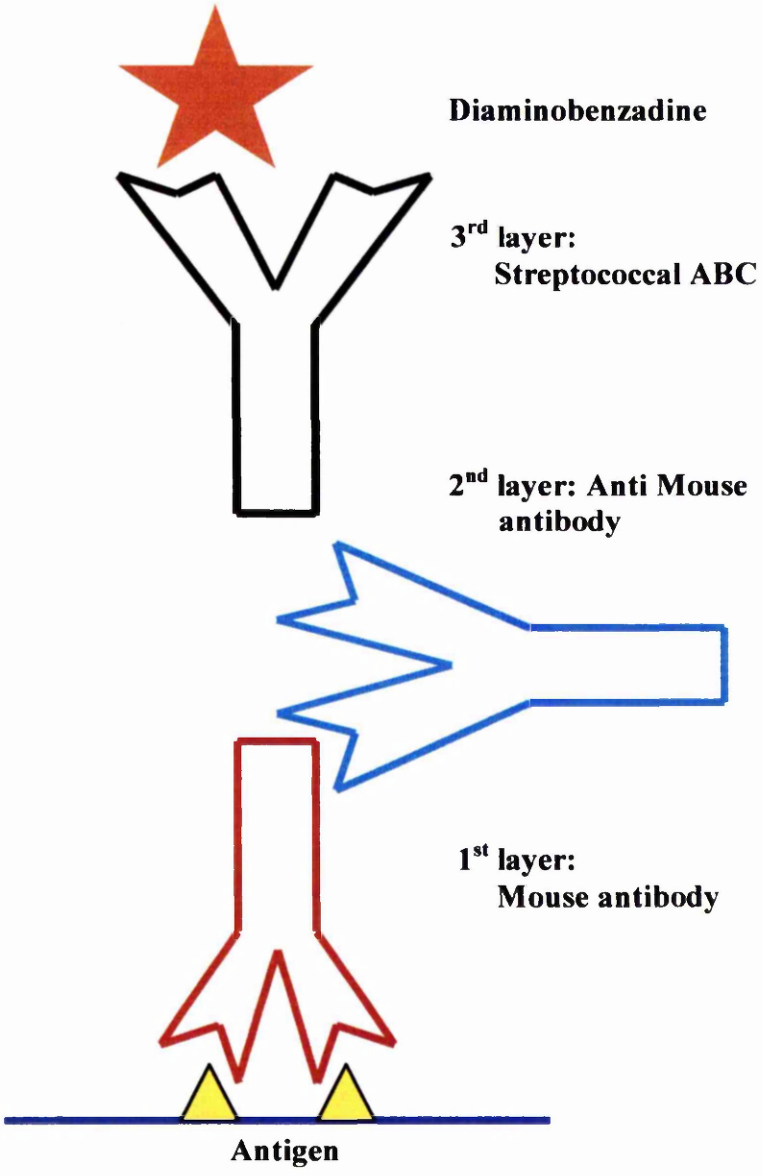


Illustration 4.2: Positive control (MNF 116-cytokeratin)

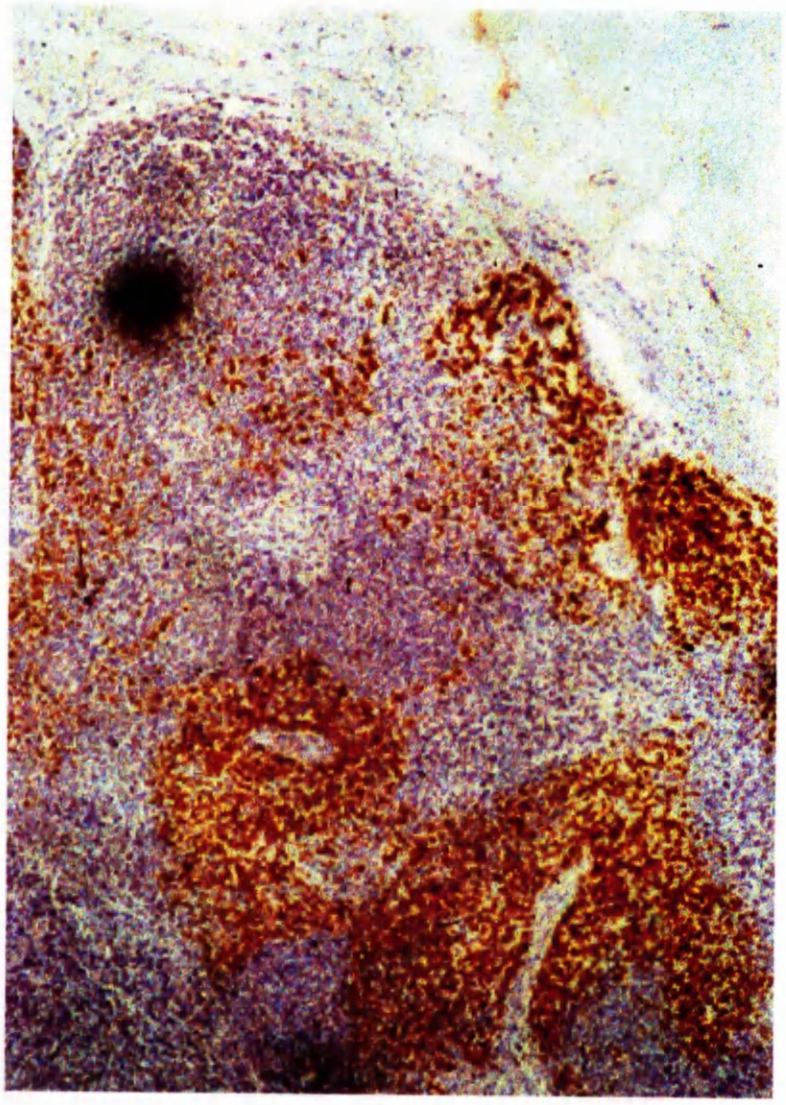


Illustration 4.3: Positive control (MUC-1 CORE)

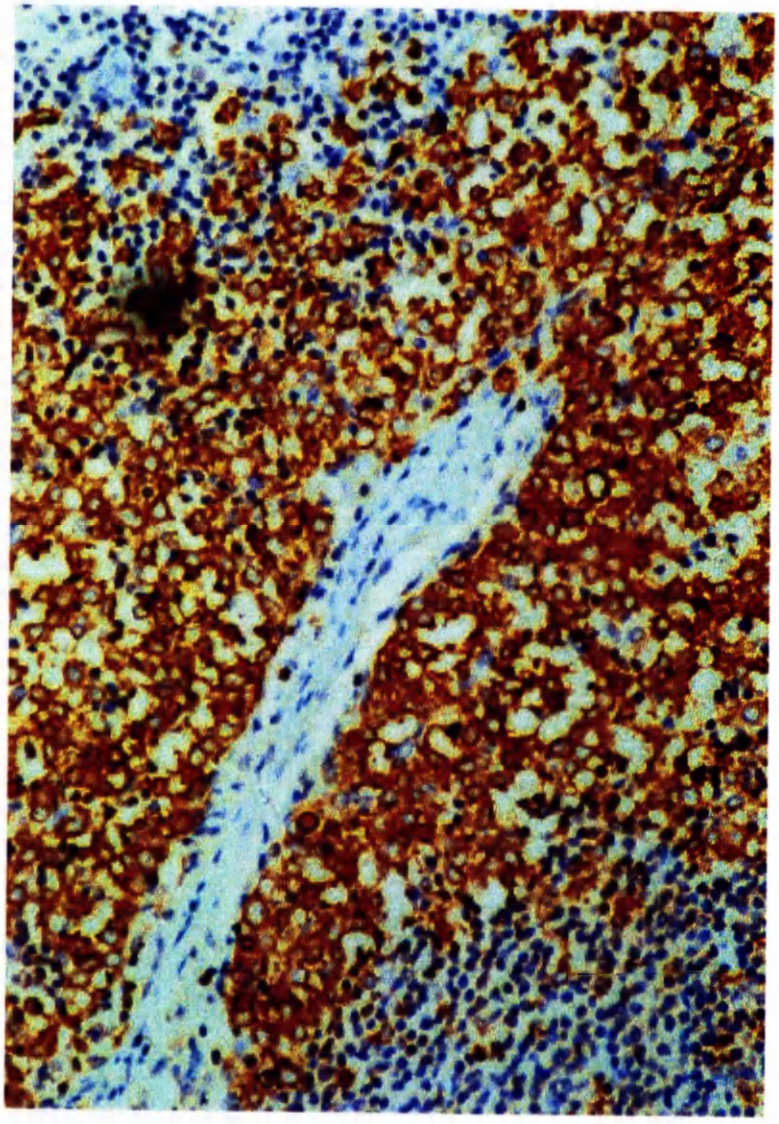


Illustration 4.4: Haematoxylin & Eosin stained node with micro-metastasis

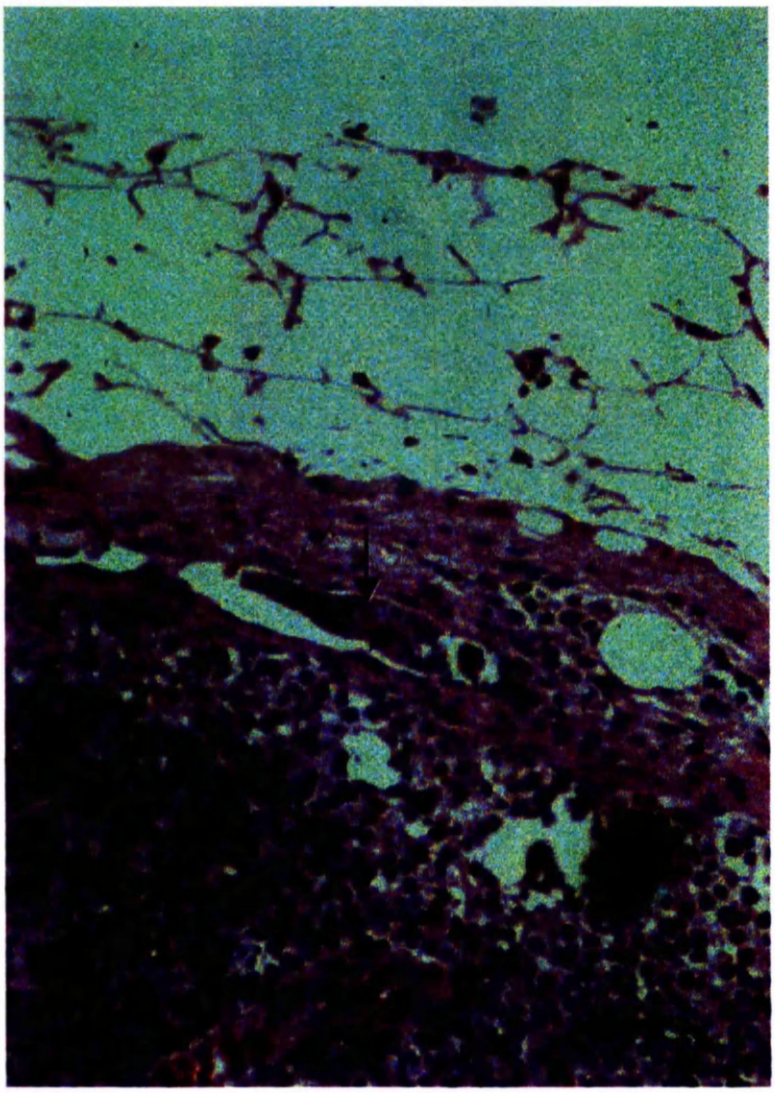


Illustration 4.5: MNF 116-cytokeratin stained micro-metastasis

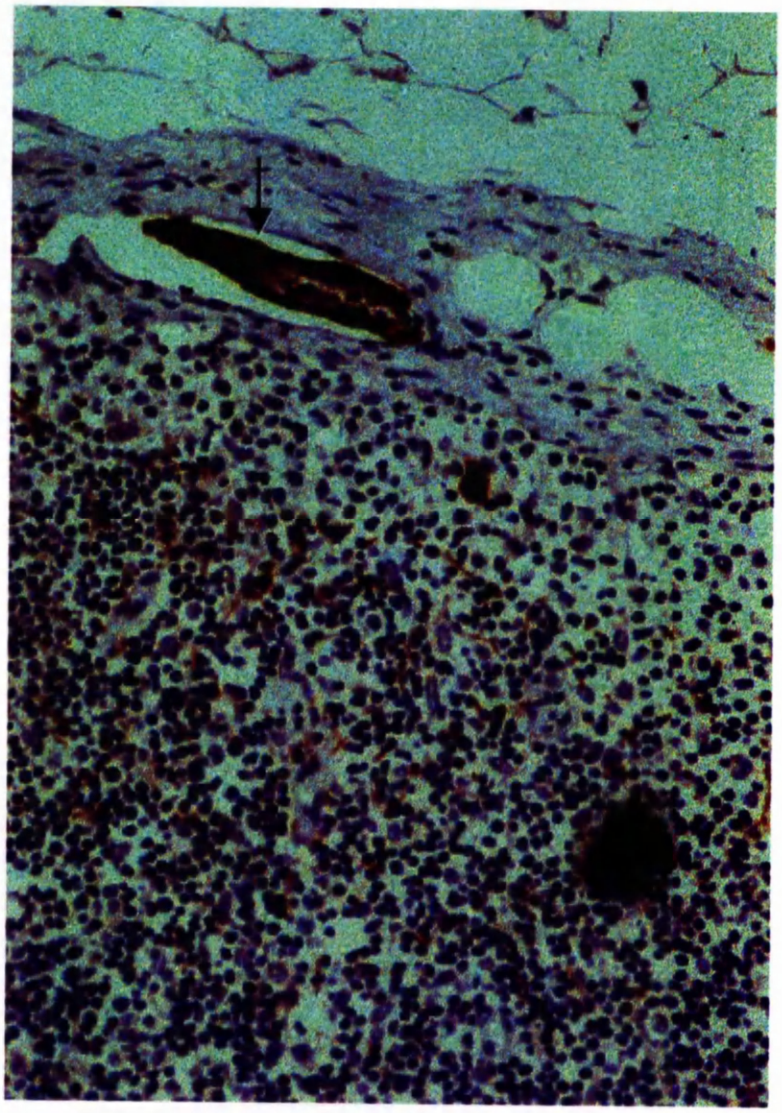


Illustration 4.6: MUC-1 CORE stained micro-metastasis

RESULTS

The mean age of patient was 55 years (range 42-69 years, S.D. 7.18 years). The mean number of axillary nodes retrieved was 10 (range 2-30 nodes). Of the tumour bed positive patients in the study group 5 (50%) patients had invasive disease within the cavity shavings and the remaining patients had residual in-situ disease only.

Results of patient pathology and underlying differences between the study and control groups are represented in Table 4.1.There was no significant difference between the study and control groups for age at diagnosis; pathological tumour diameter; oestrogen receptor status; tumour grade or type.

Table 4.1: Clinico-pathological factors

	Study group	Control group
Mean age (years)	56	51 p=0.108
Invasive ductal carcinoma	18 **(95)**	19 **(100)**
Lobular carcinoma	1 **(5)**	0
Grade I	0	1 **(5)**
Grade II	9 **(47)**	8 **(42)**
Grade III	9 **(47)**	10 **(53)**
Not defined	1 **(6)**	
Mean tumour diameter (mm)	15.6	14.6 p=0.102
Oestrogen receptor positive	8 **(42)**	10 **(53)** p=0.089
Tumour bed positive	10 **(53)**	9 **(47)**
Total numbers of patients in each column	**19**	**19**

- Values in parentheses are percentages

Clinico-pathological factors and occult axillary disease

This study identified that only 2 (5.6%) of the patients had occult axillary disease, Table 4.2. Due to these small numbers with occult axillary disease, no statistical associations could be made between clinico-pathological factors and tumour bed positivity with occult axillary disease. It is worthy of note however, both patients with occult axillary disease were tumour bed positive.

Table 4.2: Clinico-pathological features of patients with occult axillary disease

Clinico-pathological features	Patient 1	Patient 2
Age (years)	58	43
Tumour type	Invasive ductal carcinoma	Invasive ductal carcinoma
Tumour grade	II	III
Tumour size (mm)	6	30
Oestrogen receptor status	Positive	Negative
Cavity shaving status	Positive (invasive disease)	Positive (in-situ disease only)
Outcome	Well	Systemic recurrence

DISCUSSION

The development of metastatic disease is a complex process involving a series of selective events, all of which depend on the complex interaction between host and tumour factors (202). One factor being recently highlighted as an independent predictor of poorer survival is the presence of residual disease within the tumour bed (186). Furthermore, the effect of tumour bed positivity on poorer outcome seemed to be independent of the presence of residual disease or nodal status (186). These findings suggest that patients with a positive margin have aggressive tumours. This finding has important implications for clinical practice as tumour bed positivity is found in 37% of patients undergoing breast-conserving surgery (61).

The traditional methods of staging patients with breast cancer are relatively insensitive at detecting the presence of occult disease. The use of IHC or PCR techniques to detect tumour specific markers are more sensitive methods detecting the presence of micro-metastases within the axilla and have been found to detect occult disease in up to 33% of cases (190-192,194-197,203). Sentinel node biopsy is a new technique that uses the combination of staining with blue dye and radioisotope labelling to detect the first draining node from a tumour. This technique combined

with IHC or PCR may one-day lead to a highly efficient method of detecting occult disease within the axilla (204-215).

The aim of this study was to detect any association between tumour bed positivity and occult axillary disease. Such a finding would correlate well with Macmillan's results (189). In this study a total of 38 patients with 392 separate node were analysed for the presence of occult disease by the combination of serial sectioning and IHC. The expected detection of occult disease as assessed by IHC would be approximately 20 % (190-192). In this limited series however only 2 (5.6%) patients were found to have occult disease. There was no association found between tumour bed positivity and occult disease within the axilla. Furthermore, only 1 of the 19 patients who developed systemic recurrence had occult disease within the axilla. The major limitation of this study however is the small numbers of patients that were available for analysis. However, these results do show that within this institution the traditional method of nodal staging missed disease in only 2/392 nodes. This result is better than the published data where serial sectioning detected missed foci of disease in up to 15% of cases (200). This finding may be explained in part by the fact that within this institution a specialist pathologist reports on all breast cancers.

The results from this study highlight the need for a larger trial to be conducted in order to determine the exact incidence of occult nodal disease in patients with positive margins as a whole. An advantage of such a study would be that with follow-up data the effect of occult axillary disease on patient outcome could be analysed separately.

In summary, this study has not demonstrated any association between tumour bed positivity and the presence of occult disease within the axilla. These results are however due to the small numbers of patients included in this study. The results from this study further highlight the complexity of the metastatic process seen in breast cancer.

CONCLUSION

With the better understanding of cancer biology as well as the development of safer forms of radiotherapy the management of operable breast cancer has progressed from radical forms of mastectomy to simple mastectomy and finally to breast-conserving surgery. Several trials have shown that breast-conserving surgery is a safe alternative to mastectomy (15,16,38). The aim of breast-conserving surgery in the treatment of breast cancer is to excise the tumour completely and achieve an excellent cosmetic result. This must not however compromise the degree of local control allowed by mastectomy. The decision to opt for breast-conserving surgery is based on several factors including tumour size; site and patient preference. The consequences of inappropriately selecting patients for breast-conserving surgery are, re-operation (re-excision or mastectomy) in order to obtain disease-free excision margins or at worst, local recurrence.

Local recurrence following breast-conserving surgery is a matter of continuing clinical concern. The reported incidence of local recurrence is 0.3 to 15% (17,38,62-64). Furthermore, local recurrence has been reported in up to 43% of patients not receiving post-operative radiotherapy (38). The presence of local recurrence not only

undermines patient morale but may also predict for poorer survival. When patients who suffer a local recurrence are analysed as a separate population and their outcomes assessed, it has been noted that the relative risk of developing systemic disease increases to between 1.2 to 6.6 (32,38).

In the NSABP-B06 trial when a time dependent multivariate analysis was performed the presence of local recurrence was found to be a predictor of systemic recurrence (38). Veronesi et al found similar results when they reviewed their experience of 2233 patients (32). In the NSABP-B06 trial it was shown that patients who had a local recurrence occurring within 1 year of the initial surgery had a higher incidence of systemic recurrence (38). Similarly, Veronesi showed the relative risk of developing systemic disease to be 6.6 if the local recurrence had occurred within one year of surgery (32). This risk fell to 2.2 and then to 1.2 for local recurrences occurring firstly 2 and then 3 years following surgery (32). Furthermore, analysis of results of the OCOG trial has shown that local recurrence is associated with poorer survival (62). Similarly, Chauvet et al showed that the 5-year survival was 87.5% for those with local recurrence compared to 91.3% for those without (115). These results have been repeated in other studies (31,116-126).

Many risk factors for the development of local recurrence are recognised including larger tumour size; the presence of EIC; lymphovascular invasion and oestrogen receptor negativity. However, one of the most important risk factors is inadequate tumour excision. A number of different factors make the completeness of surgical excision difficult to determine. Firstly lumpectomy specimens usually have a large and irregular surface area, containing a non-uniform tumour, thus making analysis difficult. Furthermore, the pathologist commonly has to deal with specimens containing eccentric tumour extension (159). All these problems make gross assessment of tumour margin unreliable.

Several techniques have been developed to aid in the pathological assessment of the tumour margin. However, the two main techniques are "inking" and "tumour bed assessment". The technique of inking has been previously described and found to detect a positive margin in approximately 10% of cases (38). A previous study from this institution has found that tumour bed assessment detects a high incidence of residual disease (61). Furthermore, although these two techniques are not wholly comparable, inking has been found to be inaccurate at predicting the presence of residual disease as detected by tumour bed assessment (162).

This thesis deals with five main issues. These are the incidence of tumour

bed positivity; the clinico-pathological and mammographic risk factors for tumour bed positivity; the effect of tumour bed positivity on patient outcome; the effect of a policy of tumour bed assessment on the local recurrence rate and whether tumour bed positivity is a predictor of occult axillary disease.

In a series of 543 patients the incidence of tumour bed positivity was 37% (chapter one). The extent of this disease varied from 1 to 2 foci of in-situ disease within 1 block of tissue to extensive invasive disease throughout most of the cavity shavings. This figure correlates well with the previously published data (61). In the initial study from this institution by Macmillan et al the only clinico-pathological factor that predicted for tumour bed positivity was poorer tumour grade (61). However the results from this thesis suggest that a symptomatic presentation; poorer tumour grade; EIC and lymphovascular invasion all predict for tumour bed positivity (chapter one). Furthermore, there was an association between invasive disease within the tumour bed and axillary nodal disease (chapter one). This discrepancy is explained by the fact that in the current study there has been a larger cohort of patients analysed. Thus clinico-pathologic features that did not reach significance previously have now been proven to be predictors of tumour bed positivity.

During the time frame of this study a pragmatic policy of further surgery was followed with a proportion of patients undergoing re-excision or mastectomy. Of the 37% of patients with a positive margin 42% underwent further surgery. Of the 58% patients who were tumour bed positive but did not undergo further surgery all had between 1 to 3 foci of disease confined to one block of tissue. Interestingly in the earlier study by Macmillan et al only 35.6% of patients with a positive margin underwent further surgery compared to 42% in this updated series (61). This suggests that there has been a gradual change with time towards further surgery if a positive tumour margin is present.

In summary the study in chapter one has shown several clinico-pathological features that predict for a positive tumour margin. Furthermore, all the risk factors for tumour bed positivity have also been previously shown to be independent risk factors for the development of local recurrence (17,31,32,34-38,42). This raises the possibility that the presence of poorer tumour grade; lymphovascular invasion and EIC may be markers of a more locally advanced disease process within the breast. Consequently in such cases a traditional wide local excision could leave behind residual disease that can eventually progress on to a local recurrence.

The local recurrence rate achieved by this institution was 2% (mean follow-up 52 months) (chapter one). This figure compares favourably with the published literature (17,38,62-64). Furthermore, tumour bed positivity was found to be a predictor of both poorer DFS as well as DDFS (chapter one). These findings are similar to the previously published results (189). There was however no association between tumour bed positivity and either overall survival or local recurrence. Although patient follow-up ranged from 14 to 105 months (mean 52 months) longer follow-up may be required before any effect of tumour bed positivity on overall survival can become evident. The absence of a relationship between tumour bed positivity and local recurrence is explained by the fact that those patients with extensive disease within the tumour bed went on to further surgery in order to ensure adequate tumour clearance, thus reducing their risk. Furthermore, the relationship between tumour bed positivity and poorer outcome appears to be independent of residual disease within the tumour bed or axillary nodal status. This suggests that those patients who have extensive residual disease at the onset form a subgroup of patients who have aggressive cancers.

In order to assess the impact of implementing a policy of tumour margin assessment on the local recurrence rate, the study described in chapter

two was performed. In this study the local recurrence rate fell from 16% pre-1988 to 2.5% post-1988. This fall in local recurrence may be due to an improvement in the adequacy of local tumour excision provided by tumour bed assessment. However, these results may be biased by differences in clinico-pathological factors as well as adjuvant therapy between these two groups of patients. There were significantly more patients in the pre-1988 group that had larger tumour that were node positive and tended to be oestrogen receptor negative. Oestrogen receptor negativity has been shown to be a risk fact for local recurrence (45,46). A similar result has been found in this study. Also, larger tumour diameter has been shown to be a risk for local recurrence in both the Milan and the NSABP-B06 trials (32,16). Furthermore, differences in the extent of local surgical excision practised prior to 1988 may also have influenced the relatively high local recurrence rate witnessed prior to 1988. This is indicated by the fact that 32% of the local recurrences pre-1988 occurred within 1 year of surgery confirming inadequate tumour excision as being a major factor.

Mammography is an essential tool in the pre-operative assessment of patients undergoing surgery for breast cancer. In addition to revealing diagnostic radiological abnormalities, pre-operative mammographic

features may also determine those patients that should be suitable for breast-conserving surgery. Several studies have found a correlation between pre-operative mammographic features and clinico-pathological risk factors for the development of local recurrence (169-177). A previous publication from this institution identified that an absent mammograhic nidus; casting calcification and mammographic density were predictors of tumour bed positivity (29). The study described in chapter three found that there was an association between casting calcification; absence of a mammographic nidus; maximum nidus size as well as the ratio of maximum mammographic abnormality to breast size with tumour bed positivity. On performing a multivariate analysis however only the presence of casting calcification predicted for tumour bed positivity, relative risk of 2.7169. Unlike the previous study the presence of a dense mammographic pattern was not found to be associated with tumour bed positivity. This finding may be due to the presence of inter-observer variation as a different radiologist interpreted mammographic findings in the current when compared to the previous study. Furthermore, the larger numbers of patients analysed in the current study may also have influenced this result.

Previous studies as well as the work contained within this thesis suggest

that tumour bed positivity is a predictor of poorer survival (31,32,38,62). Furthermore, the impact on poorer outcome appears to be independent of the extent of residual disease within the breast or nodal status (189). This suggests that patients with a positive tumour margin form a subgroup of patients with aggressive disease. The advent of IHC and PCR has increased the ability to detect occult disease. In order to assess whether tumour bed positivity is associated with the presence of occult disease within the axilla the study in chapter four was performed. This study found that there was no association between tumour bed positivity and occult axillary disease. However, the relatively small numbers of cases included in the analysis limits the power of this study. Despite these results the impact of tumour bed positivity on poorer survival cannot be underestimated.

The main strength of this thesis is that the cohort of patients analysed is larger than in any previously published data from this unit. Thus several clinico-pathological risk factors of tumour bed positivity that did not reach significance previously have now reached significance. Also, the multivariate techniques used for analysis of the predictors of tumour bed positivity are strong statistical tests that have been used for the first time on this data. Furthermore, the study contained in chapter two is one of the

few studies that directly analyses the impact of tumour bed assessment on the local recurrence rate. The fixed follow-up period of 5-years used in chapter two negates the effect of differing follow-up periods on patient outcome. Despite differences between the pre- and post-1988 cohorts that may have influenced outcome, this study has shown the benefits of tumour margin assessment on reducing the local recurrence rate.

Although the work within this thesis has its merits it also has several limitations. Firstly, this data was collected in a retrospective manner from a non-randomised cohort and thus is open to bias. Furthermore, the time frame for this study is over a 12-year period during which there were changes in both surgical as well as oncological practice. Also, the pathological data in this study was not reviewed. Thus for some of the earlier patients included in this study a specialist pathologist may not have reported the pathological slides raising the possibility of inaccuracy. These factors make analysis of such data difficult and again open to bias.

The main implication on clinical practice of this thesis is that meticulous care is needed during breast-conserving surgery to ensure adequate tumour excision in order to prevent local recurrence. This work has shown tumour margin assessment to be a simple technique that detects a

high incidence of residual disease and its implementation can result in a low local recurrence rate. Furthermore, several clinico-pathological as well as mammographic risk factor for tumour bed positivity have been identified and these can be used to identify those patients that would not be suitable for breast-conserving surgery.

This thesis deals with many of the issues surrounding tumour margin assessment. However, there are several issues that require further analysis. The relationship between tumour bed positivity and poorer survival is of utmost importance as 37% of patients have disease within the tumour bed. There is the need for prospective randomised trials that assess the impact of tumour bed positivity on patient outcome as well as examining whether the use of adjuvant therapy in tumour bed positive patients alters their poorer prognosis. Furthermore, the study described in chapter four could be used as the basis for further work that determines both the incidence of occult axillary disease in patients with tumour bed positivity as well as the impact of such disease on patient outcome.

In summary the understanding of the mechanisms of local recurrence as well as its implications on patient outcome is of relevance to any clinician managing a patient with breast cancer. Anyone practising breast-

conserving surgery needs to ensure great care in achieving an adequate tumour clearance. There have been several techniques developed in order to achieve this goal of which the technique of tumour margin assessment has been shown to detect a high incidence of residual disease within the tumour bed. The implementation of a policy of tumour margin assessment combined with further surgery results in a low local recurrence rate. Furthermore, the relationship between tumour bed positivity and poorer survival has important implications, however this finding needs further investigation before changes can be made to current clinical practice. It is believed that the work contained within this thesis has helped improve our understanding of these specific aspects of patient care.

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